

**ANTAL KERPELY DOCTORAL SCHOOL OF  
MATERIALS SCIENCE AND TECHNOLOGY**



**Development and Characterization of Modified Starch Polymer and  
Polymer Composites**

**A Ph.D. dissertation submitted to Antal Kerpely Doctoral School for the  
degree of Doctor of Philosophy in the subject of Materials Science and  
Technology**

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## LIST OF ABBREVIATIONS AND NOTATIONS

10PTPS	10% Pyromellitic acid crosslinked thermoplastic starch
10TeTPS	10% terephthalic acid crosslinked thermoplastic starch
20PTPS	20% Pyromellitic acid crosslinked thermoplastic starch
20TeTPS	20% terephthalic acid crosslinked thermoplastic starch
5PTPS	5% Pyromellitic acid crosslinked thermoplastic starch
5TeTPS	5% terephthalic acid crosslinked thermoplastic starch
T	Absolute Temperature.
ASTM	American Society for Testing and Materials
CCTPS	Cured Citric Acid Crosslinked Starch
CA	Citric Acid
CaTPS	Calcium carbonate precipitate filled thermoplastic starch
CTPS	Citric Acid Crosslinked Starch
3CTPS	Crosslinked starch by citric acid produced at pH<3
HCTPS	Cross linking was done at a pH of 12 and then neutralized to 7 pH
CLOCS	Crosslinked oxidized cassava starch
NCTPS	CTPS without curing (non-cured CTPS)
Td	Decomposition temperature
DSC	Differential Scanning Calorimetry
DMA	Dynamic Mechanical Analysis
Ea	Energy of activation
ECH	Epichlorohydrin
FTIR	Fourier Transform Infrared Spectroscopy
Tg	Glass transition temperature
IUPAC	International Union of Pure and Applied Chemistry
LDPE	Low density polyethylene
MaTPS	Malic acid crosslinked thermoplastic starch
MO	Malonic acid
MoTPS	Malonic Acid crosslinked Thermoplastic Starch
Tm	Melting temperature
NST	Native corn starch
NCTPS	Non-cured; citric acid, starch film sample produced at pH ≈6
WS	Non-plasticized starch
OA	Oxalic acid
OTPS	Oxalic acid crosslinked starch

OCS	Oxidized cassava starch
POCl <sub>3</sub>	Phosphoryl chloride
PE	Polyethylene
PLA	Polylactic acid
PSGG	Potato starch and guar gum
A	Pre-exponential factor
PTPS11	Pyromellitic acid crosslinked starch @pH 11
PTPS3	Pyromellitic acid crosslinked thermoplastic starch @pH 3
PTPS5	Pyromellitic acid crosslinked thermoplastic starch @pH 5
PMDA	Pyromellitic dianhydride
SEM	Scanning Electron Microscope
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
CS	Starch cross linked with citric acid, no glycerin added
WS	Starch film from water and starch suspension
E'	Storage Modulus
SA	Succinic acid
STPS	Succinic acid crosslinked thermoplastic starch
TA	Tartaric acid
TaFS	Tartaric acid crosslinked Enset fiber reinforced starch
TaMcS	Tartaric acid crosslinked montmorillonite clay filled starch
TaTPS	Tartaric acid crosslinked starch
TaTcS	Tartaric acid crosslinked termite clay filled starch
TPA	Terephthalic acid
tan $\delta$	Loss factor ( $E''/E'$ )
TGA	Thermogravimetric analysis
TPS	Thermoplastic starch
ATPS	TPS film prepared in methanol
R	Universal gas constant
XRD	X-ray diffraction

## Chapter 1

### Introduction

The use of plastic has been controversial for a long time due to the shortage of facilities or the infrastructures to recycle and its non-sustainable use, non-renewability, non-biodegradability, and its toxic additives incorporation [1]. Approximately 300 million tons of synthetic polymers are produced worldwide every year. Since polymers are extremely stable, their degradation cycles in the biosphere are practically unlimited. Environmental pollution by synthetic polymers, such as waste plastics and water-soluble synthetic polymers in waste water has been recognized as a major problem. Plastics and polymers are an integrated part of our daily existence. However, because of stability and resistance to degradation, these are accumulated in the environment at the rate of about 8% by weight and 20% by volume of the landfills.

Current trends indicate that steady growth will occur within the use of biodegradable plastics with increasing accessibility of appropriate materials due to social group and legislative pressure [2,3]. Biodegradable polymers are materials whose chemical and physical properties deteriorate and totally degrade when exposed to microbes, aerobic, and anaerobic processes. Some of the current attempts to solve the problem are focusing on mixing plastic materials with natural biodegradable polymers like starch, cellulose, and polylactic acid (PLA) which are also cheap [4–6].

The exhaustion of mineral resources like oil and also the increasing waste accumulation are the forces behind the quick growing interest in biopolymers. Biopolymer describes on one hand polymers that are biodegradable and on the other hand plastics synthesized from renewable sources. Ideally, these properties are unified within the same material. However, such ideal biopolymers still suffer from poor mechanical properties, a short fatigue life, lesser resistance to chemicals, lesser durability, and restricted processing capability and that especially limit their applications and potential to replace conventional polymers [7–9].

Starch is a readily available biopolymer derived primarily from grains and tubers that is completely biodegradable [10–12]. Being renewable and biodegradable, starch has been seeking the attention of many researchers worldwide to replace the environmentally unfriendly conventional plastics [13–18]. A number of physical, and chemical modification techniques has been utilized to modify starch [19]. Variety of chemical agents are used to chemically modify starch film. Cross-linking, Grafting polymerization, Oxidation, Etherification, Esterification are among the chemical modification methods of starch [19].

Research and improvement in polymeric materials coupled with a suitable filler, matrix-filler interaction and modern techniques to create composites have potential applications in food bundling. Advancement in food bundling/packaging materials is anticipated to develop with the appearance of cheap, renewable and economical materials with improved barrier and mechanical properties [20].

Biodegradable materials are being continuously and exponentially modified and improved in all fields of use [21–26]. Crosslinking is one of the most popular methods to alter the behavior of biodegradable starch polymers [27,28]. Crosslinking or cross-linkage is interconnection of linear or branched chains in a polymer. Ether or ester bonds are formed when the reagents react with the hydroxyl groups in starch molecules crosslinking process [29]. This modification increases the polymer's rigidity by forming a three-dimensional network. Crosslinking in starch increases the degree of polymerization and molecular mass; starch molecules lose water solubility and become soluble in organic solvents. Several agents are used to crosslink native starch: sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (ECH) and phosphoryl chloride ( $\text{POCl}_3$ ), among others [30–33]. Polysaccharides containing hydroxyl groups have the possibility to be crosslinked by polyfunctional carboxylic acids. Examples of poly-carboxylic acids that have been used to cross-link polysaccharide materials are 1,2,3,4-butanetetracarboxylic acid [34, 35], poly (maleic acid) [35] and citric acid [34]. Some examples of polysaccharide materials which have been crosslinked with polycarboxylic acids are starch granules [36], starch nanoparticles [37], starch films [38], starch gels [39].

Significant number of researches are done on modification of starch using crosslinking agents, fillers and reinforcements, but there is still a huge gap in replacing the conventional plastics by bioplastics. This study basically concentrates on the limitation of researches involving the many easily available and non-toxic carboxylic acids which can potentially replace the toxic plastics.

With a purpose of improving mainly the water resistivity and related physical and mechanical property of starch biopolymer, plasticizers (glycerol), cross linking agents (carboxylic acids), fillers (clays), and natural fibers (Enset) was used to modify the physical and chemical nature of starch using crosslinking, pressure moulding and solution casting techniques.

## Chapter 2

### Theoretical Background and Literature Review

Theoretical background and literature are reviewed based on starch, starch based biodegradable polymers, starch modification techniques, starch modification agents, fillers, and natural fibers. Starch film processing, crosslinking of starch, testing, and characterization and application of starch based polymers and composites are particularly introduced and addressed in this chapter.

#### 2.1 Starch based Biodegradable Polymer

Biodegradable materials are employed in a variety of applications, including packaging, agriculture, and medicine. Biodegradable polymers have received much interest in recent years. There are two types of biodegradable polymers: synthetic and natural polymers. Polymers can be made from feedstocks generated from either petroleum resources (nonrenewable resources) or biological resources (renewable resources). Natural polymers have generally better benefits than synthetic polymers [40].

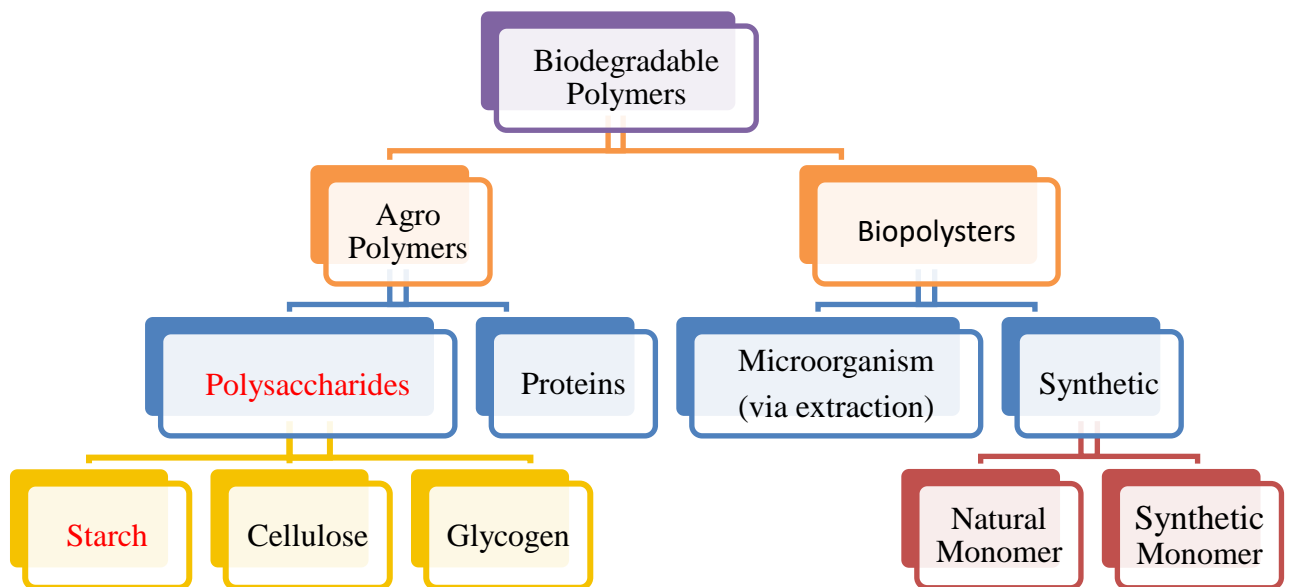


Figure 2. 1 Classification of biodegradable polymers

### **2.1.1 Biodegradable Polymers from Biological Sources**

In recent years, biodegradable polymers derived from renewable resources have received a lot of interest. This renewed interest stems from increased worldwide environmental consciousness and the challenge of fossil fuel depletion. Recently, biopolymer research and development, as well as production, has been the most rapidly growing study area [41].

Biodegradable polymers from renewable sources can be found in agricultural sources or animal sources like Bacteria. Proteins and polysaccharides like starch are among the natural biodegradable polymer sources. In this study, starch, specifically corn starch is used as a source for further modification.

## **2.2 Starch Modification and Application**

Starch is a hydrocolloid biodegradable polymer, which is considered to be a class of carbohydrate as it contains carbon, hydrogen, and oxygen of ratio 6:10:5 [42]. Starch is a polymeric carbohydrate composed of anhydroglucose units, which are deposited in plant tissues as insoluble semi- crystalline granules that vary in shape, size, and structure, depending on their origin. It is mostly composed of a mixture of two biopolymers (glucans): amylose (straight chain) and amylopectin (branched chain). The structure of the starch granule depends on how amylose and amylopectin are associated and distributed [43]. The ratio between amylose and amylopectin varies depending on the starch source. In regular starches, amylose constitutes about 15–30% of total starch [44].

The final properties of starch would get affected when there is a change in concentration of these compounds, namely amylose and amylopectin. Elongation strength of starch would increase when amylose content increases [45].

Amylose is essentially a linear structure of  $\alpha$ -1, 4 linked glucose units fig. 2.1a. A large number of hydroxyl groups in its structure confers hydrophilic properties to the polymer, resulting in a material with a high affinity for moisture. Because of its linear nature, mobility, and the presence of many hydroxyl groups along with polymer chains, amylose molecules tend to orient themselves parallel to each other and approach each other near enough to allow the formation of hydrogen bonds between adjacent chains. It is widely accepted that the most favorable three-dimensional conformation for amylose is a double helix, which can pack into regular arrays [46].

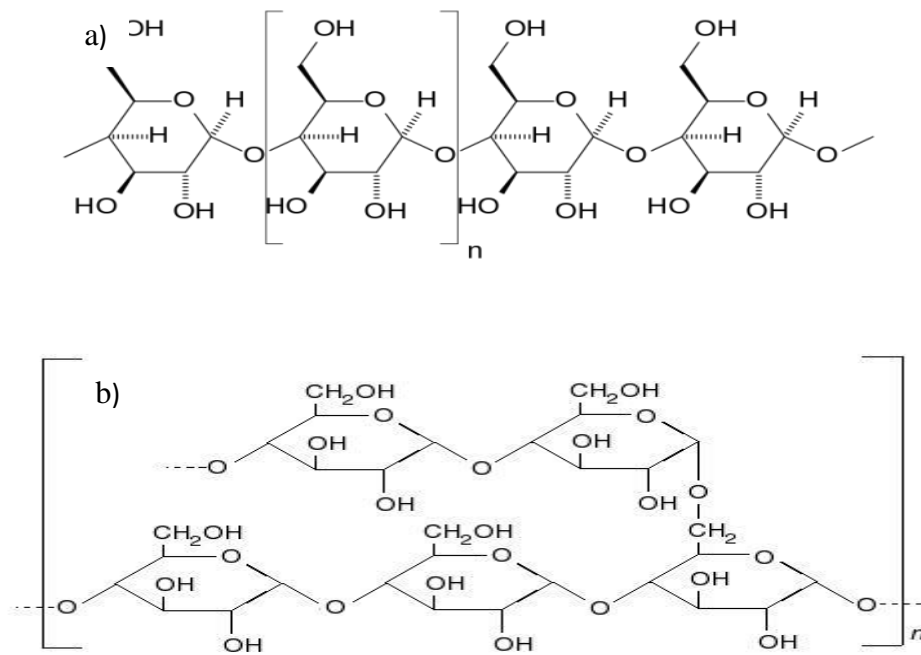


Figure 2. 2 Chemical structures of (a) amylose and (b) amylopectin [47]

Amylose also referred to as a linear polymer, which is one of the two main components of starch. Amylose can be described as a linear polysaccharide in which anhydroglucose units are linked by  $\alpha$ -D-1, 4 glucosidic bonds. It forms about 20-30% of the starch structure in most common starch sources.

Amylose content and branch length and placement in amylopectin are the major determinant factors of starch functional properties, such as water absorption, gelatinization and pasting, retrogradation, and susceptibility to enzymatic attack [48]. According to their origin, starches possess differing amylose and lipid-complexed amylose contents, amylopectin chain-length distributions, relative crystallinity, microstructures, swelling behavior, gelatinization properties, and pasting/rheological characteristics. Due to their strong and flexible structure, transparency (derived from the linear structure of amylose), and resistant to fats and oils, starch films are useful for numerous applications in the food industry. Their functional properties depend on the source but they are also affected by other factors like chemical modification, system composition, pH, and ionic strength of the media [49].

Crops that are high in starch are the principal source of food calories for people globally, although many affluent people tend to think of starch as a thickener for sauces and gravies or as “bad carbs” in a health-conscious diet. On the other hand, even technologically savvy people might not mention starch as a potential raw material for making environmentally friendly plastics. Nevertheless, starch is renewable, readily biodegradable, easily modified both



physically and chemically, and available in bulk in all parts of the world at low cost (estimated global production >70 million tons in 2011), making it a very attractive raw material for the manufacture of “green” plastics [50].

Many varieties of starch have less than 15% amylose amount in their granule makeup. These starches are named waxy starches due to waxy look in their endosperm [51]. These starches need high amount of energy for gelatinization due to the availability of a high amount of amylopectin that owns high crystallinity in its structure [52]. Waxy starch requires high energy for gelatinization due to its high crystallinity [53].

Cereal starches have lipid molecules in their structures in the form of phospholipids and free fatty acids; they are proportional to the amylose ratio [54]. The existence of lipid complexes in starch granules is detected as a hydrophobic nucleus positioned within helices made by amylose chains. The amount of lipid complexes in cereal starches was found to be between 0.15 to 0.55% of the amount of amylose [54]. The small amount of lipids in starch granules, is found to considerably lessen the swelling capacity of the starch paste [55]. Starch has about 0.6% of protein with in the molecule. Proteins and lipids are positioned on the surface granules of starch. The availability of lipids and proteins in starch granules has a significant effect on their functionality. Starch also contains a relatively small quantity (<0.4%) of minerals calcium, magnesium, phosphorus, potassium and sodium). Among these, phosphorus is of very important and is existing in starch in three main forms: monophosphate esters, phospholipids and inorganic phosphate.

Amylose is anhydrous and can make very good films, the films are colorless, strong, odorless and tasteless, and this important behavior of amylose makes it to have several applications in industries [56].

Corn starch: one main difference between corn varieties is the proportion of amylose and amylopectin that ranges from 2% amylose (waxy corn) to over 90% amylose. The normal corn variety has an amylose content of 24-28% of amylose and 75% amylopectin [57]. Environmental factors have high impact on corn starch and its structural features during its phase of development as a plant [58]. The peak gelatinization temperature was highly affected by the location of the planting. Corn starch grown in tropical environment were found to have narrower gelatinization temperature than those grown in temperate environment [59].

Starch is hardly usable in its native state due to its fragility and low mechanical property which leads to poor film forming capacity. These defects are overpowered by plasticization and blending with other polymers [60].

The scientific and business community finds in starch a good alternative for biodegradable packaging applications, as it is a renewable resource widely available that can be obtained from different agricultural surplus and industrial leftovers from raw materials processing [61]. Besides, among all biodegradable polymers, its cost is relatively low. Starch naturally occurs in a variety of botanical sources such as cereal grains (corn, wheat, and rice), seeds, legumes (lentils), and tubers (potato and cassava). In addition, it is completely compostable without toxic residues, odorless, tasteless, colorless, nontoxic, and biologically absorbable [62]. Many different reactants are used for starch esterification. Among them, acetic, maleic, malonic acid, succinic, and octenyl succinate anhydrides, dodecyl succinate, and tosyl chlorides can be used. Overall, starch esterification is known to increase the hydrophobic behavior, decrease the retrogradation tendency, and increase the compressional and flow properties of starch [63].

### **2.2.1 Processing of Thermoplastic Starch**

Starch is processed similarly to the processing of conventional synthetic thermoplastics [64]. Those include solution casting, internal mixing, extrusion, injection molding, and compression molding [65]. One vital issue to be considered in the processing of starch is the presence of water [66]. Starch processing is critically supported by water and/or plasticizers. The decomposition temperature ( $T_d$ ) of dry starch is less than the melting temperature [67]. One important problem that could influence the mechanical properties of the final material is water evaporation during starch processing, which generates instabilities and occluded bubbles [68]. The materials processed exclusively with water are usually brittle. For that reason, not easily evaporated plasticizers, such as polyols, are employed [33–36].

#### **2.2.1.1 Starch Plasticization**

Plasticizers are mostly liquid organic compounds that are not very volatile. They improve the polymer's versatility, extension, toughness, and processability when incorporated into a polymer or elastomer. The mechanical and thermal properties of biofilms are enhanced by the use of various plasticizers. Plasticizing is most common method because it gives the formulator flexibility to use different formulations. Improvements in elongation, softness, solvency, surface lubricity, decreased viscosity, improved thermal stability, and flexibility are all benefits of using the plasticizer [73].

Water is considered a primary plasticizer, it changes the structure of the molecular bonds of starch upon heating. Water content affects the oxygen permeability of starch bio-plastic film. At low humidity conditions, films are excellent barriers against oxygen transmission [74]. Even if water is the main plasticizer, it is not always wise to use water as a sole plasticizer of TPS. Less volatile plasticizers like polyols, glycerol, pentaerythritol, poly(oxyethylene)s, poly(oxypropylene)s, citric acid, sugar alcohols, non-ionic, and anionic surfactants are also used. At low concentrations and depending on water content; plasticizers with a high affinity for water, like glycerol, will exhibit anti-plasticization at particular concentrations. When the added plasticizer has a higher affinity to water than starch, the plasticizer water coupling lowers the amount of plasticizer available for hydrogen bonding with starch, thus anti-plasticization occurs. This leads to a rise in the gelatinization temperature, GT, and increased brittleness [75]. According to a study assessed using glass transition temperature, the anti-plasticization limit for glycerol was found to be ~10–15% w/w, and increases for xylitol, as its anti-plasticization limit reaches 20% w/w [39–41].

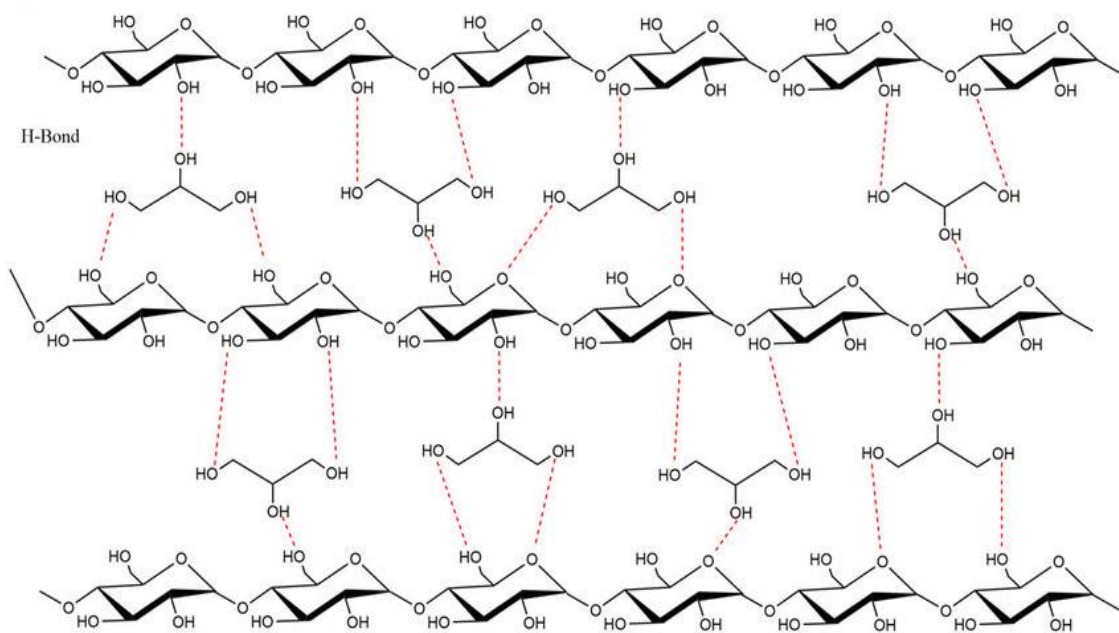


Figure 2. 3 Plasticization of starch using glycerol [79]

The decomposition temperature and boiling point of pure glycerol is at 290°C and has a melting point of 17.8°C. Glycerol has three hydroxyl groups in its structure and is hygroscopic, tending absorbing moisture from the air [80]. Glycerol has polyol groups that can create hydrogen bonds with water, so, glycerol is easily soluble in water and can form an aqueous solution. The

availability of three hydroxyl groups of glycerol permits reactions with various organic acids to form esters [80].

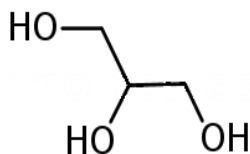


Figure 2. 4 Molecular structure of glycerol

### 2.2.2 Crosslinking of Starch Using Carboxylic Acids

Compared to inorganic or mineral acids, carboxylic acids are weaker acids. Malonic and glutaric acids are examples of organic acids with lower molecular weights that show good solubility in water. Higher molecular weight acids, on the other hand, are essentially insoluble in water but have excellent solubility in organic solvents. Depending on the acid type and the conditions of the reaction, carboxylic acids can be used for starch cross-linking, esterification, hydrolysis, and grafting processes. The developments of starch modification using carboxylic acids (oxalic acid, citric acid, malonic acid, succinic acid, tartaric acid, pyromellitic acid, terephthalic acid, and malic acids) are introduced in this review. The modified starch's change in physicochemical and structural characteristics are also discussed.

#### 2.2.2.1 Crosslinking of Starch Using Succinic Acid

Succinic acid is a naturally occurring four-carbon dicarboxylic acid produced by liquefied petroleum gas [81]. However, petroleum gas is expensive and thus succinic acid (SA) is generated by different microbes. SA is naturally formed by most living cells as an outcome of anaerobic digestion. It is a common organic acid, which can be used in many foods, chemicals, and pharmaceutical industries as a precursor to generate many chemicals such as solvents, perfumes, lacquers, plasticizer, dyes, and photographic chemicals. Succinic acid is also used as an antibiotic and curative agent. It also finds application as a surfactant, ion chelator, and as additive in various industries [82].

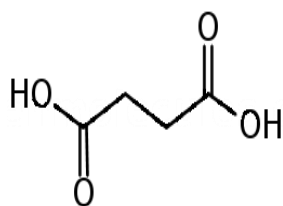


Figure 2. 5 Molecular structure of succinic acid

The cross-linking of granular starch with SA was an effective way to enhance viscosity stability of cooked OCS (oxidized cassava starch) paste. Viscosity sensitivity of cooked OCS paste to heat and shear could be reduced with a low level of SA cross-linking of starch. The cross-linking introduced covalent bonds between starch molecules. As a result, CLOCS (cross-linked oxidized cassava starch) was more resistant to heat and shear than OCS. In addition to stabilizing the viscosity, the cross-linking showed substantial influences on fundamental performances such as water dispersibility, adhesion-to-fibers, film properties, and desirability. SA treated starches showed B type crystalline pattern with diffraction intensity of retrogradation peak ( $2\theta = 16.7$ ) increasing with number of retrogradation cycles. Up to 0.15% of SA, the viscosity of the modified cassava starch was improved; however, as the SA concentration increased, the light transmittance decreased, Because SA filled in the gaps in the starch network. Starch films created after cross-linking with SA has higher tensile strength and thickness up to an optimum concentration [46,47].

Potato starch and guar gum composite film incorporating organic acids (succinic acid, malic acid, and tartaric acid) having two different functional groups (carboxyl and hydroxyl groups) showed that esterification reactions occurred between PSGG (potato starch and guar gum) molecules and the organic acids. This reaction improved structural compatibility between polymeric phases. This also increased stretch ability and transparency of the films, while the tensile strength and water barrier property of the films were diminished significantly compared to native PSGG film. Incorporation of acids into PSGG composite film also increased solubility and water absorption capacity. Therefore, it can be inferred that even though the organic acids were blended with PSGG composite film as a cross-linker, it acted as a plasticizer for PSGG composite system [85]. SA modified starch showed an increase in the molecular weight of starch chains. Besides, the molecular weight increase was similar for amylose and amylopectin fractions. This suggests that OSA could be acting as a cross-linking agent between starch chains, reducing the susceptibility to amylolysis [86]. SA treatment induced the formation of more complex starch chains, offering more resistance for amylolytic reactions [87].

Under alkaline pH conditions, wheat starch was exposed to a SA acid acetanhydride mixture (4.6 % and 8 % w/w; SA acid; acetanhydride 3.225 %) for 30 minutes before being neutralized. The total reaction time was given as 2 hours. It was discovered that the carbonyl group of esters is represented by the FTIR band at  $1740\text{ cm}^{-1}$ . With an increase in reagent concentration, treated wheat starches displayed an increase in gel strength. However, it was discovered that as reagent concentration increased, paste clarity and freeze-thaw stabilities tended to deteriorate. The

decreased gelatinization temperature of the modified starch was attributed to the reduction in crystallinity during modification. Due to the amylopectin's rearrangement under the influence of heat and solvent, as well as the reduced amylose content, the modified starch has demonstrated increased swelling and solubility [88, 89].

In a study that used adipic, succinic, and oxalic acids to react with maize starch to form starch films using casting technique. The thermal behavior of the starch films revealed greater interaction between succinic acid and starch. The films made of oxalic acid and adipic acid were less crystalline than those made of corn starch. A halo indicating an amorphous material was seen from the XRD pattern of the succinic acid-containing film. The FTIR spectra revealed the ester formation, which demonstrates the interaction of the acid with starch. According to the study, succinic acid was the most reactive with starch indicating the impact of the size of the acid chains on the thermal and structural characteristics of biofilms [90].

Films made of wheat starch and hydroxypropylated corn starch (70:30) that were crosslinked with concentrations of SA (0.38-0.75% w/w) displayed higher mechanical strength and low water vapor permeability. SA acid cross-linked films appeared smoother with voids under field emission scanning electron microscopy, which may be related to the interfacial compatibility. Due to the esterification and transesterification reactions between SA acid and starch, FTIR spectra showed the appearance of a peak at  $1715\text{ cm}^{-1}$  in modified films. The polymeric films had a whitish appearance and a capacity to adsorb water by more than 120% [91].

#### 2.2.2.2 Crosslinking of Starch Using Malic acid

Malic acid (MA) is an organic compound that falls under the category of dicarboxylic acids because it has two  $\text{-COOH}$  groups at two terminals. There are two stereoisomers of malic acid: L and D isomers. But only L isomers exist naturally. The preferred IUPAC name of this compound is 2-Hydroxybutanedioic acid [92]. The chemical structure of this compound is as follows:

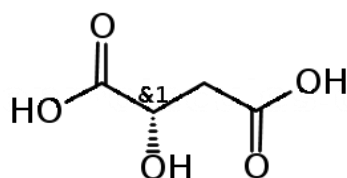


Figure 2. 6 Molecular structure of malic acid

Malic acid has been vastly researched for its crosslinking use of starch for packaging purposes [61]. Malic acid crosslinked cassava starch-PVA blend has improved water-resistance properties. The cross-linked starch film and the same film with the addition of cellulose filler showed an increase in strength and tensile resistance, however, the crosslinked bioplastic with the addition of palmitate cellulose filler showed a decrease in stretchability [93]. Cassava starch crosslinked with malic acid has shown a change in the intra and intermolecular hydrogen interactions in the grafted starch when compared to native starch. The formation of di maleate esters has enhanced the hydrophobicity of the grafted starch [94].

Hydroxyl groups of corn starch react with malic anhydride to form ester linkages, which eventually form a three-dimensional network structure. Peeling test experiments show that the adhesion force of the cross-linked corn starch binder is 4.9 times higher than that of the corn starch binder without cross-linking [95].

Sweet potato starch treated with MA (0.5-2.0 M) demonstrated that the starch granules remained intact at all MA concentrations used. MA concentrations were found to cause an increase in degree of substitution (DS), and carbonyl peaks ( $1722\text{ cm}^{-1}$ ) were shown on the FTIR as the result of esterification. At higher reaction pH and temperature, the granule shape became less uniform from the granules. The XRD pattern showed that there was little to no destruction of the starch's crystalline structure, which can be attributed to the leaching of amylose. It's possible also that long chains of starch were reduced to short chains and then rearranged into crystal structures [96].

In a related study, corn starch was added to MA (50% w/v) for 16 hours at a pH of 1.5, dried to 5–10% moisture, and heated for 5.5 hours at  $147^{\circ}\text{C}$ . Malate starch was discovered to be white in color, but as DS increased, the starch's whiteness, pasting viscosity, and crystallinity decreased [97].

In a study that used MA (20% w/w) to modify wrinkled pea starch; starch suspensions (25%) was mixed with MA, which was then treated with heat and moisture (HMT) for 6 hours at  $140^{\circ}\text{C}$  while being kept at an acidic pH for 24 hours. As a result, the majority of the hydroxyl groups present were esterified during the treatment, leaving fewer free hydroxyl groups available to interact with water molecules and reducing the swelling power and solubility of modified starch. The swelling power of each modified starch sample decreased, and FTIR measurements revealed a new peak at  $1731\text{--}1741\text{ cm}^{-1}$ . Despite having a relatively unchanged crystal structure, the crosslinking of the chains and the steric hindrance of the attached MA molecules make it difficult for water molecules to penetrate between the chains [98].

Wheat starch suspension (40% w/w) was mixed with acids (150 mg/kg starch basis) and kept at 25°C for 30 minutes before being neutralized, washed, dried, and sieved. The granules' surface was colored with spots as a result of both acids, according to scanning electron microscopy. The acids made the samples more water soluble while decreasing intrinsic viscosity and water absorption. Malic acid significantly decreased the temperature and enthalpy of gelatinization, while citric acid had no significant impact on these parameters, according to the analysis of the thermal properties. The starch gels' hardness, adhesiveness, elasticity, and cohesiveness were all reduced by both acids. Malic acid was generally more effective than citric acid in influencing the functional properties of starch [99].

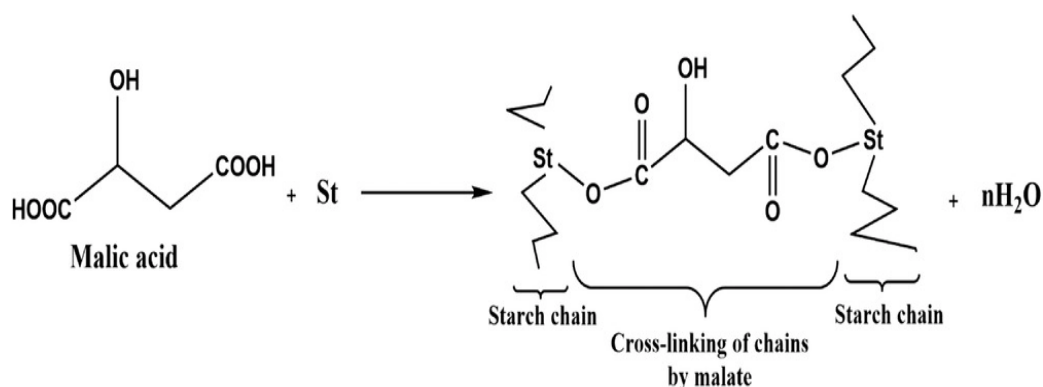


Figure 2. 7 Crosslinking of starch using malic acid [100]

### 2.2.2.3 Crosslinking of Starch Using Malonic acid

Malonic acid (MO) belongs to the class of organic compounds known as dicarboxylic acids and derivatives. These are organic compounds containing exactly two carboxylic acid groups. MO is a plant-derived organic dicarboxylic acid with a molecular weight of 104 g/mol. It is a non-toxic acid and is soluble at room temperature, it is also capable of reacting with hydroxyl groups on the D-glucopyranose ring present in many polysaccharides [101]. Figure 2. 5 below shows the molecular structure of malonic acid.

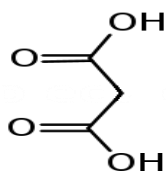


Figure 2. 8 Molecular Structure of Malonic acid

Malonic acid has been used in the surface modifications of polymer films. Chemical, thermal and mechanical test results confirmed the crosslinking, as well as reduced moisture sensitivity of corn starch crosslinked with malonic acid [102], Crosslinking of potato starch using malonic



acid has increased physical and mechanical properties [103]. The use of malonic acid as a crosslinking agent for starch has effectively improved the mechanical property at significantly higher degrees of substitution [104].

In a study that examines the impact of malonic, citric, succinic, and oxalic acids on the physical properties of starch-based polymer films, the authors came to the conclusion that using malonic acid as a crosslinking agent enables the formation of transparent elastic starch polymer films with high surface density, low hygroscopicity, low water sorption, and high resistance to hydrolytic decomposition. However, the research doesn't explain how or why this effect occurs [105]. Figure 2.9 represents a crosslinking reaction of starch using malonic acid as a crosslinking agent and sodium hypophosphite monohydrate (Nahyp) as a catalyst.

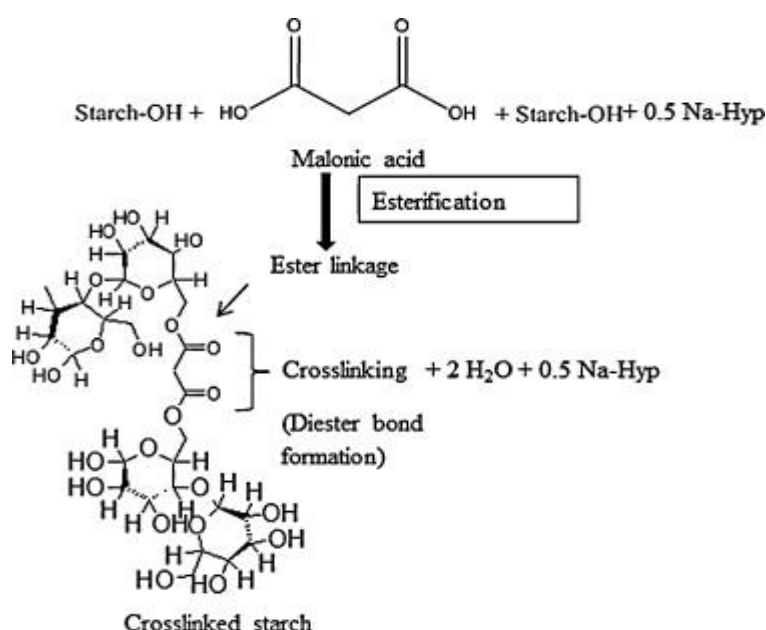


Figure 2. 9 Malonic acid crosslinked starch chemical reaction representation [102]

#### 2.2.2.4 Crosslinking of Starch Using Tartaric Acid

Tartaric acid (TA), also called dihydroxybutanedioic acid, is a dicarboxylic acid, one of the most widely distributed plant acids, with a many food and industrial uses. Along with several of its salts, cream of tartar (potassium hydrogen tartrate) and Rochelle salt (potassium sodium tartrate), it is obtained from by-products of wine fermentation [106]. Fig. 2.9 represents the molecular structure of tartaric acid.

A maximum 6% increase in tensile strength was seen in modified starch using TA as a crosslinking agent and monosodium tartrate as a catalyst under varied reaction conditions. This was thought to happen as a result of greater London dispersion forces acting inside the cross-linked starch [107].

Starches that have been chemically modified by reacting corn starch with tartaric acid-containing acetic anhydride exhibit characteristics of cross-linked starches, including decreased solubility in common starch solvents, increased resistance to the action of amyolytic enzymes, and significantly altered gelatinization behavior. The results of the characterization also clearly show the evolution of derivatization within the granule volume, demonstrating the process of cross-linking and acetyl group incorporation [108].

Tartaric acid exhibits similar effects on modified starch hydrogels as other cross-linking agents, making it a viable cross-linking agent for use in starch modification. The main benefit of TA is that it can be extracted from wine - making waste water using reasonably priced procedures [109][110].

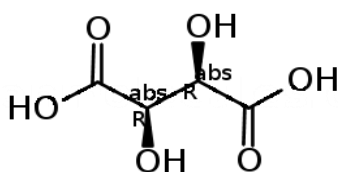


Figure 2. 10 Molecular Structure of Tartaric Acid

#### 2.2.2.5 Crosslinking of Starch Using Citric Acid

Citric acid, abbreviated as CA, that occurs naturally in the form of polycarboxylic acid is available in many different fruits, mainly in citrus fruits like lemons.

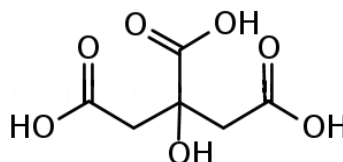


Figure 2. 11 Molecular structure of citric acid

Polysaccharides containing hydroxyl groups can be crosslinked by polyfunctional carboxylic acids. Examples of poly-carboxylic acids that have been used to cross-link polysaccharide materials are 1,2,3,4-butanetetracarboxylic acid [35,36], poly (maleic acid) [35] and citric acid [111]. Some examples of polysaccharide materials that have been crosslinked with polycarboxylic acids are starch granules [36], starch nanoparticles [37], starch films [38], and starch gels [110]. In all these studies, the reaction temperature is high, well above 100°C, and long reaction times (minutes) are used. This is because of the slow reaction kinetics of the esterification of polycarboxylic acids with hydroxyl groups on polymers due to the low amount of polycarboxylic acid used.

Citric acid can react with two or more of the hydroxyl groups present in the starch. One good reason for using citric acid as a cross-linker is due to an unreacted CA is considered nutritionally harmless and may also act as a plasticizer for starch [112].

The esterification cross-linking reaction between citric acid and starch can be performed at both acidic and basic conditions. For acidic conditions, known as Fischer esterification, the esterification reaction is an equilibrium-controlled reaction between a carboxylic acid group and a hydroxyl group. The reaction starts with protonation of the carboxylic acid group, which in turn can be attacked by the alcohol group forming an ester. In this reaction, water is produced as a by-product that limits the equilibrium unless it is evaporated [113]. The reaction can be performed both with and without additional catalysts. Often the catalyst is a strong inorganic acid. The acid used as the catalyst needs to be stronger than the poly-carboxylic acid to be esterified otherwise excessive neutralization of the catalyst will retard the crosslinking reaction. The catalyst should be able to neutralize the carboxylic acid group, but not vice versa [35,47]. It has been indicated that it is not the addition of catalysts as such, but rather that the pH was the important factor for the esterification reaction to take place [115].

Citric acid has been used for the increment of hydrophobicity of starch by crosslinking it under a dry reaction. The citric acid crosslinked starch was found to have a greater affinity for solvents of low polarity like dichloromethane. It was found that the crosslinked starch nanocrystals displayed a higher affinity for solvents of low polarity such as dichloromethane [116].

Adding citric acid to biodegradable starch has improved thermal stability, water resistance, and fluidity due to the strong hydrogen bond created between starch and citric acid. Citric acid has negatively influenced the tensile stress and has also prevented starch from recrystallization and widens the processing temperature of starch [117]. Citric acid is also reported to increase the plasticity of starch by first depolymerizing the starch granules when added with water and glycerol [118]. Hydrolysis of starch solution in the presence of citric acid is highly influenced by citric acid concentration, reaction temperature, and pH during crosslinking [119].

Structural properties of the reaction chemicals affect both the reaction rate as well as the equilibrium. Primary alcohols are more readily esterified than secondary alcohols, which in turn are more easily esterified than tertiary alcohols due to steric reasons. The reaction rate in acidic conditions is proportional to both the reactants and the  $H^+$  ion concentration. The equilibrium constant of the esterification reaction is dependent on the temperature and the presence of salts. Usually, to achieve completion of the esterification equilibrium reaction either the water or the ester is removed from the reaction mixture. In a laboratory scale,

sulphuric and hydrochloric acid is often used as the classical catalyst for acid-catalyzed esterification reactions. Phosphoric acid also works as catalyst but yields slower reaction kinetics.

Citric acid has two pathways for the esterification of hydroxyl groups, both the Fisher esterification and an anhydride formation mechanism. When citric acid is heated, it dehydrates yielding a highly reactive cyclic anhydride that easily reacts with the hydroxyl groups in the starch.

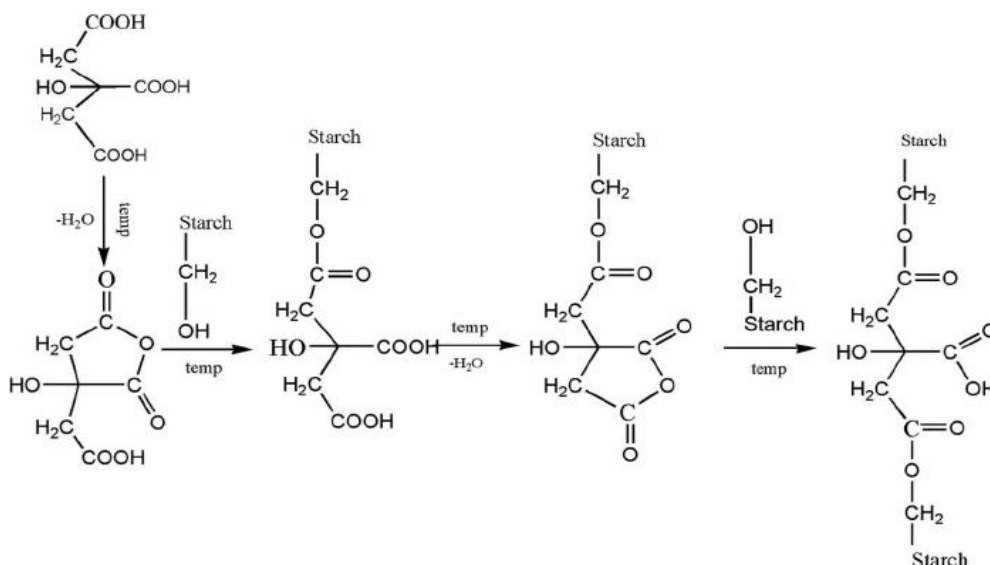


Figure 2. 12 Crosslinking of starch using citric acid [120]

### 2.2.2.6 Crosslinking of Starch Using Oxalic Acid

Oxalic acid is a colorless powder or granular solid with no odor, is nonvolatile and has a high water solubility  $220 \text{ gL}^{-1}$  at  $25^\circ\text{C}$ . Under environmental conditions (pH 5–9), it is in the form of oxalate ion ( $\text{p}K_{\text{a}1}$  and  $\text{p}K_{\text{a}2}$  of 1.25 and 4.28, respectively). Oxalic acid and its complexes are biodegraded in aerobic and anaerobic conditions in soil and water in less than one day. Thus, very low concentrations of oxalic acid in water sources and food crops are expected. In the atmosphere, removal from the air via dry/wet deposition and photolysis is likely to occur [121].

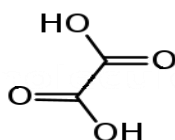


Figure 2. 13 Molecular structure of oxalic acid

According to studies, transparent elastic starch polymer films with a high surface density and low thickness can be produced by crosslinking starch using oxalic acid. These films also exhibit low hygroscopicity, little water absorption, and high hydrolytic degradation resistance [122, 123].

### 2.2.2.7 Crosslinking of Starch Using Terephthalic Acid

Terephthalic acid (TPA) is a high-volume commercial chemical that is primarily manufactured through the aerobic catalytic oxidation of para-xylene (pX), which is derived from fossil fuels. TPA is principally utilized as a co-monomer in the condensation of polyethylene terephthalate (PET) with mono ethylene glycol (MEG) to generate PET for plastic containers, fibers, films, and other applications [124]. The polymer is a high-melting, crystalline material forming very strong fibers.

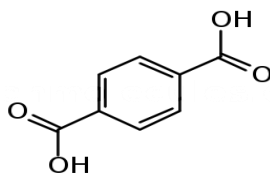


Figure 2. 14 Molecular Structure of Terephthalic Acid

In a study is aimed at understanding the role of cross linker geometry on the properties of PVA. PVA solution (5% w/v) was prepared by dissolving PVA in N,N dimethylformamide (DMF) was mixed with suberic or TPA. The crosslinking was done under similar reaction conditions and concentration. It is observed that crosslinking of PVA by suberic acid is more effective than TPA in improving tensile and thermal properties. The maximum strength measured in crosslinked samples is 32.5 MPa for suberic acid crosslinked PVA which is higher than that of neat PVA (22.6 MPa). Swelling study shows that 8 h crosslinked TPA (35% w/w) samples have a minimum of 5.4% of water uptake compared to neat PVA. FTIR spectroscopy confirms the formation of crosslink ester bond in crosslinked PVA [125].

### 2.2.2.8 Crosslinking of Starch with Pyromellitic Dianhydride

Pyromellitic acid dianhydride (benzene 1,2,4,5-tetracarboxylic dianhydride): Pyromellitic dianhydride (PMDA) is an organic chemical with the formula  $C_6H_2(C_2O_3)_2$ . The double carboxylic acid anhydride is utilized in the production of polyimide polymers like Kapton. It is a hygroscopic white solid. It condenses into a hydrate. This substance is a member of the organic compound class known as tetracarboxylic acids and derivatives. These are carboxylic acids that have four carboxyl groups.

Pyromellitic acid dianhydride (PMDA) is a key cross-linking agent utilized in the production of high-temperature-resistant polymers. Because of the functionality group features, its commercial uses are expanding. One of the most significant applications is the production of polyethylene terephthalate (PET) for food packaging materials [126].

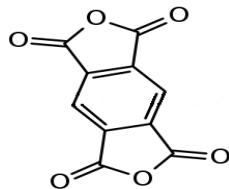


Figure 2. 15 Molecular Structure of Pyromellitic dianhydride

The primary influencing factor on pyromellitic dianhydride modified Potato starch was found to be reactive temperature using orthogonal tests. A degree of substitution of 11 was observed in a product prepared at 40°C for 3 hours with a mass ratio of 16:1 of starch and dianhydride and a pH value of 7.5. The distinctive absorptions of the C = O and COO- groups were represented by the peaks at 1750 cm<sup>-1</sup> and 1586 cm<sup>-1</sup>, respectively, which the author described it as the successful formation of starch ester [127].

### 2.2.3 Thermoplastic Starch Polymer Composites

Polymer composites possess better properties as compared to neat polymers without sacrificing density, toughness, and processability [128]. In addition, biopolymer-based materials manufactured using green technology has shown biodegradability and biocompatibility in pharmaceutical, food packaging, and agriculture technologies [129].

#### 2.2.3.1 Thermoplastic Starch Reinforced with Natural Fibers

To increase TPS's low mechanical and physical properties, such as its low resistance to stress and moisture, cellulose fibers can be used as reinforcements. Processing of TPS reinforced with cellulose fibers is similar to that of the majority of conventional synthetic thermoplastics. Most thermoplastic reinforcing techniques use heat and pressure to generate desired shapes. The TPS reinforced with cellulose fibers can be processed using the same methods as thermoplastics. Extrusion, injection molding, internal mixing, compression molding, and other processes are some of the techniques often used. Through continuous research over the last few years, Natural fiber-reinforced composites have considerably developed in mass. Currently, extensive research is taking place worldwide on natural fibers and their composites to increase their properties.

Choosing natural fibers over synthetic fibers improves biodegradability while lowering health and environmental dangers. Furthermore, the similar chemical nature of plant fibers and starch allows for good compatibility between matrix and reinforcing fiber. Because cellulose fibers had higher crystallinity and thermal resistance than starch, adding cellulose fibers or microfibers to TPS improved the biocomposite's water resistance and thermal resistance [130].

Fibers are categorized as natural or man-made fibers. Due to their low cost, low density, and low tool wear, natural fibers have substituted man-made fibers in various applications. In addition, their renewability and sustainability has brought them to be used widely and highly utilized in the composite field. Based on their origins, Natural fibers are classified into three types namely plant, mineral, and animal fibers. Mineral and animal fibers have not been used widely in composites as reinforcement. However, numerous plant fibers have been utilized broadly in the biocomposites field for applications in the areas of automotive, packaging, marine, and construction. Regenerated cellulose fibers that are categorized under natural and man-made fibers have been utilized as reinforcement fibers in the latest times [131].

Natural fibers have a different chemical composition that leads to variation in their properties, so; detailed comparison is needed while selecting them for use. Plant fibers, particularly bast and leaf, find applications in various industries while most of the other fibers are being explored in on a lab scale but haven't found large scale commercial applications [131]. Among the natural fibers available, we have used Enset fiber in our study.

**Ensete Fiber:** is a natural cellulosic fiber extracted from the Ensete plant (*Ensete Ventricosum*), The Ministry of Agriculture and Natural Resources of Ethiopia reported that Enset is one of the important indigenous food crops where an estimated 15-20 million people mostly in the Southern region, parts of Oromia region and Gambela States support livelihoods as a staple food. The pulp is fermented for food production but its strong fibers are now only used for local rope making or they are discarded. Jointly, selling the fibers for manufacturing uses would be a win-win situation for both the industries and the farmers, and provide the latter with a much needed source of additional income because composite material reinforced with Ensete fibers can be used for diverse industrial application including the production of parts for automotive industry and packaging products.

A study comparing the effect of raw and chemically treated oil palm mesocarp fibers as reinforcement for TPS found that raw fiber reinforced composite materials had better intermolecular interactions and mechanical capabilities than those reinforced with treated fibers. The interaction of the van der Waals force between the silica group of fiber and the -OH group of starch could have caused this [132]. Biodegradable trays prepared from cassava

starch and agricultural residues such as sugarcane bagasse, cornhusk, malt bagasse, and orange bagasse demonstrated improved rigidity and complete biodegradation in 60 days when compared to expanded polystyrene trays. During storage at high or medium relative humidity, all of the biodegradable trays demonstrated a high water sorption capacity [133].

Short cellulosic fibers from bleached pulp were employed as reinforcement in a study that used *Eucalyptus urograndis* to enhance the mechanical properties of corn starch. The composites were made with ordinary cornstarch that had been plasticized with glycerin. In a powerful batch mixer operating at 170°C, the fibers were directly added to the thermoplastic starch. The mixture was hot pressed into plates that were 2-4 mm thick. Comparing the composite to non-reinforced thermoplastic starch, tensile strength increases by 100% and modulus increases by more than 50% [134].

In a study that focuses on the mechanical and thermal performance of TPS reinforced with cellulose and lignocellulose fibers. Plasticized starch matrix has been used in a variety of formulations with varied matrix composition, filler content, and fiber length and nature. DMTA study reveals significant changes in the main relaxation temperature that can be attributed to interactions that reduce the mobility of the starch chain as well as a regular reinforcing effect. In addition, it is demonstrated that the thermal resistance of these biocomposites is enhanced by the addition of cellulose fillers [135].

The ideal concentrations were found to be 12.0 wt% fiber and 24 wt% glycerol content in a research of biocomposite materials made from acetylated sugar cane fiber-reinforced acetylated corn starch in the presence of glycerol as a plasticizer. Better interactions, mechanical characteristics, and water resistance were all displayed by this concentration. The composition's elongational characteristics were enhanced by the chemical surface modification of the fiber and matrix as well as the plasticizer concentration, which led to greater matrix and fiber bonding than matrix-matrix contact [136].

*Eucalyptus* cellulose fibers were used to reinforce TPS films in a study that sought to improve its properties. Casting technique was used to create the films from suspensions of cassava starch, cellulose fibers (1.2 mm long and 0.1 mm in diameter), glycerol, and water. Three relative humidity gradient ranges, RH (2-33%, 33-64%, and 64-90%), were used to analyze the effect of fiber addition on water vapor permeability. The tensile strength and deformation capacity of films reinforced with cellulose fibers were higher, and the water vapor permeability of films was lower than that of films without fibers [137].



### **2.2.3.2 Thermoplastic Starch Composites Reinforced with fillers**

TPS has a higher degree of wettability, which restricts its use. A lot of research has been done on the integration of fillers such talc, calcium carbonate, and clays to enhance the product's characteristics and expand its applicability. As they operate as a stress concentration in the system, adding micro fillers as reinforcement to the biopolymer matrix may result in a decrease in the mechanical characteristics of the bio-composites. This could be mitigated by utilizing methods to decrease coagulation and reducing the particle size of fillers [138].

Clay minerals are hydrous silicates described as fine-grained particles with sheet-like structure piled over each other. Due to this geometry, they are usually named as phyllosilicates, sheet-structured silicates. Phyllosilicates are made-up of fine-grained aluminosilicates and are formed as a consequence of chemical weathering of silicate minerals at the surface of the earth [139]. Clays are commonly dominated by phyllosilicates and may be separated from the clay fraction or the bulk clay material by different approaches.

Silicates, particularly layered silicates (phyllosilicates) like montmorillonite, talc, and kaolin, are the most prevalent types of clay. Clay has an impact on the composite films' mechanical, structural, and barrier properties [140].

### **2.2.4 Application of modified starch polymer and composites**

Crosslinked starch polymers are finding extensive use in daily life due to their wide range of sources, non-toxicity, biocompatibility, and biodegradability.

In the food industry they are used for edible wrapping, tubing, or food packaging, etc. In agriculture, they are used for mulch films, fertilizers, and greenhouse coverings are used. In tissue engineering and Drug delivery systems they are used as bone tissue engineering scaffold, drug delivery system in the form of microsphere or hydrogel etc.

And generally could replace some of the non-biodegradable, non-renewable petroleum-based polymers used for things like flower pots, cups, and toothbrush handles.

### 2.3 Literature Summary and knowledge gap

Due to its renewability, biodegradability, low cost, and abundance of -OH chemistry, which opens it up to endless modification possibilities and melt processability in the presence of plasticizers, starch, a renewable polysaccharide polymer, has received extensive research and commercial interest as a feedstock. However, due to limitations in its hydrophilicity, thermal, and mechanical properties, rapid degradability, and strong intra- and intermolecular hydrogen bonding of the polymer chains, it cannot be widely used in industry as a renewable biopolymer. Therefore, modification is required to alleviate these limitations and create desirable properties. Granules of starch in an aqueous suspension can react to create cross-linked starch. The hydrogen bonds in the granule are reinforced by cross-linking, which forms chemical bonds that act as molecular bridges. Since the cross-linking process involves treating the starch while it is still in its granular form, the amount of chemical cross-links added to the starch is often quite small when compared to the starch's weight and the total number of anhydroglucose units in the granule. The majority of cross-linked starches will have 1 cross-link every 100–3000 anhydroglucose units.

The reaction conditions for starch might be extremely variable depending on the temperature and the specific reagent used for the cross-linking. Alkali sodium hydroxide is frequently used to accelerate the process. Commonly, neutral to mildly alkaline conditions are used to conduct the reactions. Sometimes the reactions also take place in an acidic environment.

The majority of researches reviewed revealed most of the studies undertaken are on regularly used acids and their derivatives, including acetic anhydride, succinic anhydride, and citric acid. Nevertheless, this analysis of the literature has revealed that various carboxylic acids and their derivatives have a significant potential for producing modified starches.

The complexity of modifying starch is reported to be very high, and by altering just one reaction parameter, a new product with totally distinct characteristics might be produced. This has paved the way for many researcher to devotedly work starch cross-linking using carboxylic acids. There is no question that starch modification using carboxylic acids and their derivatives will garner even greater attention in future studies given the accessibility and affordability of starch and carboxylic acids.

Positive outcomes have been observed as a result of the work done on TPS modification using natural fillers and fibers. Cotton, sugar palm, and cassava are some examples of natural fiber reinforcements that enhance mechanical properties but have a negative impact on water resistance.

Clay is another filler that has increased water resistance but has the drawback of lowering mechanical property, according to some studies. This suggests that TPS composites, if further investigated, may soon replace petroleum-based polymer composites.

### **2.3.1 Knowledge gap**

Many studies are being conducted to physically and chemically modify corn starch. One of the chemical modification techniques, crosslinking, enhances the properties of starch-based polymers, but the majority of cross-linkers either have negative effects on the functionality of biopolymers or are toxic.

In order to produce starch based biopolymeric materials with the desired properties, green cross-linking agents and more new crosslinking techniques are required. As a result; Cross-linking of starch with various cross-linking agents have been studied; however, there is still a limitation in expanding the research on more easily available and non-toxic carboxylic acids. We observed that the studies are more of on the very commonly used carboxylic acids, other potential non-toxic and cheap carboxylic acids are not studied enough, and there is no or very few attention on the aromatic carboxylic acids.

The findings of the studies reviewed on crosslinking of starch using carboxylic acids focus on optimizing the reaction conditions like molar ratio of the reagent/cross-linker, reaction temperature and pH, and some also tried to include factors like type and amount of catalyst, reaction duration and stirring speed. According to the findings, it is observed that the authors use very diverse ways of sample preparation and different reaction conditions; and came up with varying findings on the process parameters, reaction conditions and the property of the modified starch. This shows that a lot is left to be studied on crosslinking of starch so that standard and optimized production method and product quality is met.

We have also observed that the studies on modifying of starch using carboxylic acids lack to examine the effect of the molecular structure of the cross-linking agents and draw a concrete conclusion regarding why and which functional group is primarily responsible for cross-linking. Therefore, studying the effect of the intrinsic nature of the carboxylic acids; citric, tartaric, oxalic, malic, malonic, succinic, terephthalic, pyromellitic acids on corn starch cross-linking and correlating it with their cross-linking potential is worth studying.

The other modification approach is the making of starch polymer composites. Adding fillers and reinforcement fibers have been utilized to modify its properties. But, still the improved starch based polymers composites are unable to replace the conventional polymers, indicating

that much research is needed to improve the properties and reach the desirables. Consequently, investigating new polymer composites by modifying corn starch with fillers (calcium carbonate precipitate and clay) and Enset fibers which are not previously well studied was found necessary.

### **2.3.2 Specific Objectives of the study.**

- Validate and optimize process parameters for crosslinking starch polymers and polymer composites
- Investigate the effects of the intrinsic nature (Molecular structure, acidity, solubility, carbon chain length) of carboxylic acids on starch crosslinking
- Investigate the effect of the natural fibers and fillers on the physical and thermal properties of corn starch. .
- Improve the physical and chemical property of corn starch polymer film

## Chapter 3

### Experiment and Methodology

The intension of this research is to modify starch polymers using physical and chemical methods. To improve its functionalities, starch is plasticized, crosslinked, and combined with fillers and natural fibers. Among the raw materials employed are Sisal fiber, Enset fiber, pine wood fiber, Montmorillonite clay, calcium carbonate precipitate, and carboxylic acids. Among the processes employed are crosslinking and compression molding. Crosslinked and composite starch films with various cross linkers, fillers, and reinforcements were prepared as samples. This chapter describes the raw materials used, the preparation processes, the procedures and equipment used for characterization and testing, as well as the analytic and interpretation techniques employed.

#### 3.1 Materials

100% corn starch with a moisture content of 11.4% and ash content of 0.14% was obtained from HAAS Naturals. Tartaric acid (food grade) was obtained from Tarjan es Tarsa Kft. Hungary. The citric acid (food grade) was purchased from Herman Ker Bt. Hungary. Oxalic acid (99%) was purchased from Sigma Aldrich. Terephthalic acid, 99+% was purchased from thermos scientific and pyromellitic acid anhydride was obtained from Apollo Scientific. Succinic acid (99.6%) was obtained from VWR international Leuven Belgium. Malonic acid was obtained from Apollo Scientific ltd United Kingdom. Malic acid (DL-Malic acid 98%) was purchased from Alfa Aesar, Thermo Fisher (Kandel) GmbH, Germany. Pharmaceutical quality grade glycerol was purchased from Flora Vita, Kévés Béla Kft, PhEur.9.0, USP Pharmacy quality, Natural vegetable, and 99.5% pure Glycerin E422. HCl and NaOH were purchased from Sigma-Aldrich and were used as catalysts and pH adjustments during the chemical reaction. The property of the carboxylic acids used is shown in table 3.1.

**Clay and Calcium Carbonate Precipitate Filler:** Two types of clays were used as fillers for the composite preparation. Raw Sodium Montmorillonite clay was found from Miskolc and Termite clay (iron rich kaolinite clay) was brought from Ethiopia. The clays were dried and milled in a rotary miller and micro clay with 63 $\mu$ m particle size was used for this experiment.

Calcium carbonate precipitate (SOCAL U1S2) was used as a filler for the hybrid starch film composite preparation.

**Fibre:** Ensete fiber, a plant fiber brought from Ethiopia and Pine Wood fibre found in Hungary was used as reinforcements in this study.

Table 3. 1 Property of the carboxylic acids

Acid	Molecular Weight (g/mol)	pKa			Melting Point	Solubility in water (at 20 °C)
		pKa <sub>1</sub>	pKa <sub>2</sub>	pKa <sub>3</sub>		
Citric	192	3.13	4.76	6.39	153°C	1330 g/L
Tartaric	150	2.89	4.40		275°C	1330 g/L
Oxalic	90	1.27	4.28		365°C	95.5 g/L
Malic	134	3.4			130 °C	558 g/L
Malonic	104	2.83			135°C	763 g/L
Succinic	118	4.2			184°C	58 g/L
Terephthalic	166	3.51	4.82		300°C	0.015 g/L
Pyromellitic	254	1.92	2.87	4.49	285°C	15 g/L

### 3.2 Fabrication of Modified Starch Samples

#### 3.2.1 Crosslinked Starch Films Fabrication

Corn starch films were produced by first dispersing 4% (w/w) starch in water and then heating the starch dispersion in a heater at 90 °C for 20 minutes while vigorously stirring with a magnetic stirrer. Then glycerol 36% (W/W) of starch was added and heated at the same temperature while stirring for additional 20 minutes until a transparent and viscous slurry was formed (TPS processing is finished here). Subsequently, NaOH was added to adjust the pH of the dispersion. Then after, 20% by weight of the starch of carboxylic acid (crosslinking agent), was added to the dispersion under the same heating and stirring condition for another 20 minutes. HCl acid was finally used to neutralize the solution. The solution was finally poured into Teflon coated tray and kept in a furnace at 65 °C for 48 hours. Plasticized and cross-linked starch films with an average thickness of 0.3mm were peeled from the tray and prepared for experimentation.

### 3.2.2 Clay Filled Tartaric Acid Crosslinked starch film Samples Preparation

**TaMcS, TaTcS Samples Fabrication:** 5% by weight of clay (termite/montmorillonite) was added to water and rigorously stirred for 24 hours in a different cup. Another dispersion was prepared by dispersing 4% (w/w) starch in water and is rigorously stirred for 10 minutes.

The previously prepared clay suspension was added to the starch suspension (until 5% by weight of clay to starch ratio is reached) and stirred for 30 minutes at 90 °C. Subsequently, 36% glycerol to the weight of starch was added to the clay, starch, and water suspension. The starch, clay and glycerol dispersion was heated in a heater at 90 °C and was kept at this temperature for 20 minutes while stirring rigorously using the magnetic stirrer. NaOH was added to increase the pH from 6.5 to 11. Then, Tartaric acid 20% by weight of the starch was added to the dispersion, and heating was continued for another 20 minutes, finally, HCl acid was used to neutralize the solution.

### 3.2.3 Enset Fibre Reinforced Tartaric Acid Cross-Linked Samples Preparation

*TaFS Sample Fabrication:* Starch suspension was prepared by dispersing 4% (w/w) starch in water and is rigorously stirred for 10 minutes. The 2% by weight of starch fiber (Enset fiber) which was cut previously into 2mm length, was added to the suspension and stirred for additional 10 minutes. Subsequently, 36% glycerol to the weight of starch was added to the fiber, starch, and water suspension. The starch, fiber and glycerol dispersion was heated in a heater at 90 °C and was kept at this temperature for 20 minutes while stirring rigorously using the magnetic stirrer. NaOH was added to increase the pH from 6.5 to 11. Then, Tartaric acid 20% by weight of the starch was added to the dispersion, heating was continued for another 20 minutes, finally HCl acid was used to neutralize the solution.

### 3.2.4 Hybrid Composite Samples Preparation

*TaFTcS and TaFMcS Samples Fabrication:* Starch suspension was prepared by dispersing 4% (w/w) starch in water and is rigorously stirred for 10 minutes. The 2% by weight of starch fiber (Enset fiber) which was cut previously in to 2mm length, was added to the suspension and stirred for additional 10 minutes. The previously prepared clay suspension was added to the starch suspension (until 5% by weight of clay to starch ratio is reached) and stirred for 30 minutes at 90 °C. Subsequently, 36% glycerol to the weight of starch was added to the clay, starch, and fiber and water suspension. The starch, clay, fiber and glycerol dispersion was heated in a heater at 90 °C and was kept at this temperature for 20 minutes while stirring

rigorously using the magnetic stirrer. NaOH was added to increase the pH from 6.5 to 11. Then, Tartaric acid 20% by weight of the starch was added to the dispersion and heating was continued for another 20 minutes, finally, HCl acid was used to neutralizing the solution.

### 3.2.5 Preparation of TPS and TPS composites using Compression Moulding

For the preparation of TPS; 40 gram of glycerol was added to 60 gram of corn starch (2:3 mass %) in a glass beaker. Then, it was mixed manually until it is plasticized. Next, for further homogenization, the mixture in the beaker was put in the furnace at 120 °C for three minutes. Immediately after the solution is released from the furnace it was mixed by a mixer for 5 minutes at 600 rpm. The mixed solution was compressed by compression moulding machine into thin sheets of 1 mm thickness under a compression pressure of 20 MPa and a temperature of 160 °C for 5 minutes.

For preparation of composite samples; 60 gram native starch was first added with 5 grams of powdered calcium carbonate precipitate and crashed pinewood fibre (250 µm particle size) to prepare CaTPS, WTPS respectively and 5 g each of the fiber and the precipitate for HTPS, and then 40 g glycerol was added; and the procedures used to prepare TPS were followed.

## 3.3 Characterization and Testing Methodology

### 3.3.1 Water Absorption

The water absorption capacity of starch polymers and starch polymer composites was measured according to American Society for Testing and Materials (ASTM) Test Method D570: standard test method for water absorption of plastics at room temperature.

The samples were cut into small pieces (20\*20\*0.3mm), put into an oven, and dried at 110 °C for 24 hrs. Then, they were taken out and weighed immediately ( $w_1$ ). The dried samples were conditioned at 25 °C in a desiccator. The samples were then immersed in to distilled water, and their final weight was measured ( $w_2$ ) at different time intervals. The water absorption rate ( $K$ ) was calculated from the following equation:

$$K = \frac{w_2 - w_1}{w_1} * 100\% \dots\dots\dots (1)$$

Where  $K$ = is the water absorption rate in %



### 3.3.2 Fourier Transformed Infrared Spectroscopy

The FTIR spectrum of the samples was tested using the Bruker FTIR instrument (BRUKER, Germany) using the diamond disk technique. A resolution of  $4\text{ cm}^{-1}$  with a spectral range of  $4000\text{-}400\text{ cm}^{-1}$  was used. Sixty-four scans were done to collect each spectrum.

### 3.3.3 Dynamic Mechanical Analysis

Samples ( $30\times 10\times$  about  $0.3\text{ mm}$ ) were cut from the prepared starch films. Measurement was carried out in dual cantilever mode at four frequencies,  $64\text{ }\mu\text{m}$  amplitude, using the Rheometric Scientific DMA MK-III instrument. The temperature is held for  $1.0\text{ min}$  at  $-100\text{ }^{\circ}\text{C}$  and Heated from  $-100\text{ }^{\circ}\text{C}$  to  $60\text{ }^{\circ}\text{C}$  with a heating rate of  $2\text{ }^{\circ}\text{C}/\text{min}$ .

### 3.3.4 Thermogravimetric Analysis

The thermogravimetric analysis was performed using a thermal analyzer (1750 SETARAM, sestys evolution) at a heating rate of  $10\text{ }^{\circ}\text{C}/\text{min}^{-1}$  in a static oxygen environment throughout a temperature range of  $0\text{-}600\text{ }^{\circ}\text{C}$ . The sample and reference holders are placed on a common rod in a small-size tube furnace. This rod is linked to a micro-balance with four decimal precision.  $15\text{ mg}$  of each sample was placed into platinum crucibles. The test's data collection method detects temperature and mass variations in the sample when compared to the reference material (annealed alumina).

### 3.3.5 Differential Scanning Calorimetry

The heat flow measurement was made using Perkin Elmer DSC131 Evo. The thermoplastic starch composite specimens of nearly  $10\text{ mg}$  mass were heated from  $20$  to  $200\text{ }^{\circ}\text{C}$  at a rate of  $10\text{ }^{\circ}\text{C}/\text{min}^{-1}$  and then cooled at the same rate to  $20\text{ }^{\circ}\text{C}$ , waited at  $20\text{ }^{\circ}\text{C}$  for  $5\text{ minutes}$ , and finally reheated to  $200\text{ }^{\circ}\text{C}$ .

### 3.3.6 X-ray Diffraction

The crystalline structure of the samples was studied by XRD using Rigaku, MiniFlexII desktop XRD diffractometer with Cu radiation at  $60\text{ kV}$  and  $50\text{ mA}$  at room temperature. Scattered radiation was detected in the diffraction angle  $2\theta$  ranging from  $3$  to  $60^{\circ}$ . The ratio of the relative area of the crystalline peak to the total area of the diffractograms, expressed as a percentage (%), was used to determine the relative degree of crystallinities (DC), and was calculated using OriginPro 2021 software.

### 3.3.7 Scanning Electron Microscope

The surface features of the non-cross-linked and cross-linked films were observed using a scanning electron microscope. An EVO MA 10 scanning electron microscope (Carl Zeiss, Germany) was used to study the phase morphology. The equipment was operated at an acceleration voltage of 20 kV. A thin layer of gold was coated on the samples to prevent electrical charge during observation. Images were taken of the fractured surface at a magnification of 500X.

### 3.3.8 Tensile properties

The tensile properties of the starch films were determined on a universal material tester (INSTRON 5566) according to ASTM standard D 882-02. Testing was done on samples, each measuring 25mm length × 5mm width, and the respective thickness of samples averaging 0.5mm randomly cut from the cast films. Prior to testing, the samples were conditioned for 48 hours at 20±2 °C and 65±2% relative humidity to achieve the same moisture content. The INSTRON instrument was set to 10 mm/min crosshead speed, 10 kN load cell.

### 3.3.9 Hardness Test

The hardness test of the samples was done using a durometer ASTM D2240, Shore D hardness tester. Four measurements in different locations of the 1mm thick sample composite were carried out and the mean average of all the measurements was taken.

### 3.3.10 Activation Energy Calculation from Multiple Frequency DMA

#### Measurement

For determining the activation energy of the cross-linked and uncross-linked samples, the Arrhenius equation was used to draw the Arrhenius plot from the frequency and temperature relationship equation (2) below.

$$K = Ae^{-\frac{Ea}{RT}} \dots \dots \dots (2)$$

Where K is rate constant, Ea is the energy of activation of the process, R is the universal gas constant (8.314 J mole<sup>-1</sup>K<sup>-1</sup>), and T is the absolute temperature. A is a pre-exponential factor; the relaxation time extrapolated to infinite temperature

## Chapter 4

### Result and Discussion

In this chapter results of modification of starch using carboxylic acids as crosslinking agents are explored, scientifically interpreted, and discussed. Effect of the intrinsic nature of the carboxylic acids, fillers (clays), and natural fibers (cellulosic fibers) on the behavior of starch is investigated. Conclusions are drawn from the results, interpretation and discussion.

#### 4.1 Crosslinking of Starch Using Citric Acid

This experimental work is designed to crosslinking citric acid with different processing approaches and inputs to improve its water resistivity and its mechanical and thermal properties. Table 4.1 shows the samples produced in the experiment and the description of preparation.

Table 4. 1 Modified Starch Film Samples Prepared

S.No.	Designation of Samples produced	Description
1	CS	Starch cross linked with citric acid, no glycerin added
2	TPS	Thermoplastic starch, without citric acid
3	HTPS	Cross linked thermoplastic starch with citric acid as a cross linker; and NaOH and HCl acid as pH adjustment. Cross linking was done at a pH of 12 and then neutralized to 7 pH
4	CCTPS	CTPS (citric acid crosslinked thermoplastic starch) prepared by adding starch, water, glycerol, NaOH and citric acid. Reaction was done at a pH of 6 and cured under a furnace at 165 °C for 10 minutes (cured CTPS)
5	NCTPS	CTPS without curing (non-cured CTPS)
6	3CTPS	Cross linked TPS with citric acid. Reactions held at a pH of 3. Without adding NaOH and HCl, no pH adjustment
7	ATPS	TPS film was prepared. The film was put in a tray having a solution of 3:7 citric acid to methanol alcohol ratio. The film in the solution was put in furnace for 48 hours at 75 °C
8	WS	Starch film from water and starch suspension

### 4.1.1 Fourier Transform Infrared Spectroscopy

Fig. 4.1, shows the FTIR spectra of the samples (non-cross linked, and cross linked starch samples) with citric acid under different techniques. The FTIR spectrum of all the samples shows characteristic IR bands of starch at  $572\text{ cm}^{-1}$ ,  $830\text{ cm}^{-1}$ ,  $994\text{ cm}^{-1}$ ,  $1076\text{ cm}^{-1}$ ,  $1149\text{ cm}^{-1}$ ,  $1336\text{ cm}^{-1}$ ,  $1411\text{ cm}^{-1}$ ,  $1643\text{ cm}^{-1}$ ,  $2925\text{ cm}^{-1}$  and a broad band at  $3278\text{ cm}^{-1}$ . Two of the characteristic bands mentioned  $994$  and  $1076\text{ cm}^{-1}$  could be due to the C-O bond stretching band [141]. And  $1149\text{ cm}^{-1}$  can be attributed due to the asymmetric vibration of C-O-C bonds, which shows the formation of ether bonds that decreases the hydrophilicity of starch. The band  $2925\text{ cm}^{-1}$  is due to the symmetric and asymmetric vibration of C-H bond. The broad band  $3278\text{ cm}^{-1}$  is assigned to the hydroxyl groups of starch. Among the seven samples tested FTIR spectra of  $1723\text{ cm}^{-1}$  appeared on CS, CCTPS, NCTPS, and 3CTPS samples, while the same spectra is not seen on the samples TPS, ACTPS, and HCTPS samples. The availability/appearance of  $1723\text{ cm}^{-1}$  on CS, CCTPS, NCTPS, and 3CTPS samples shows the formation of ester groups as a result of crosslinking [142][143].

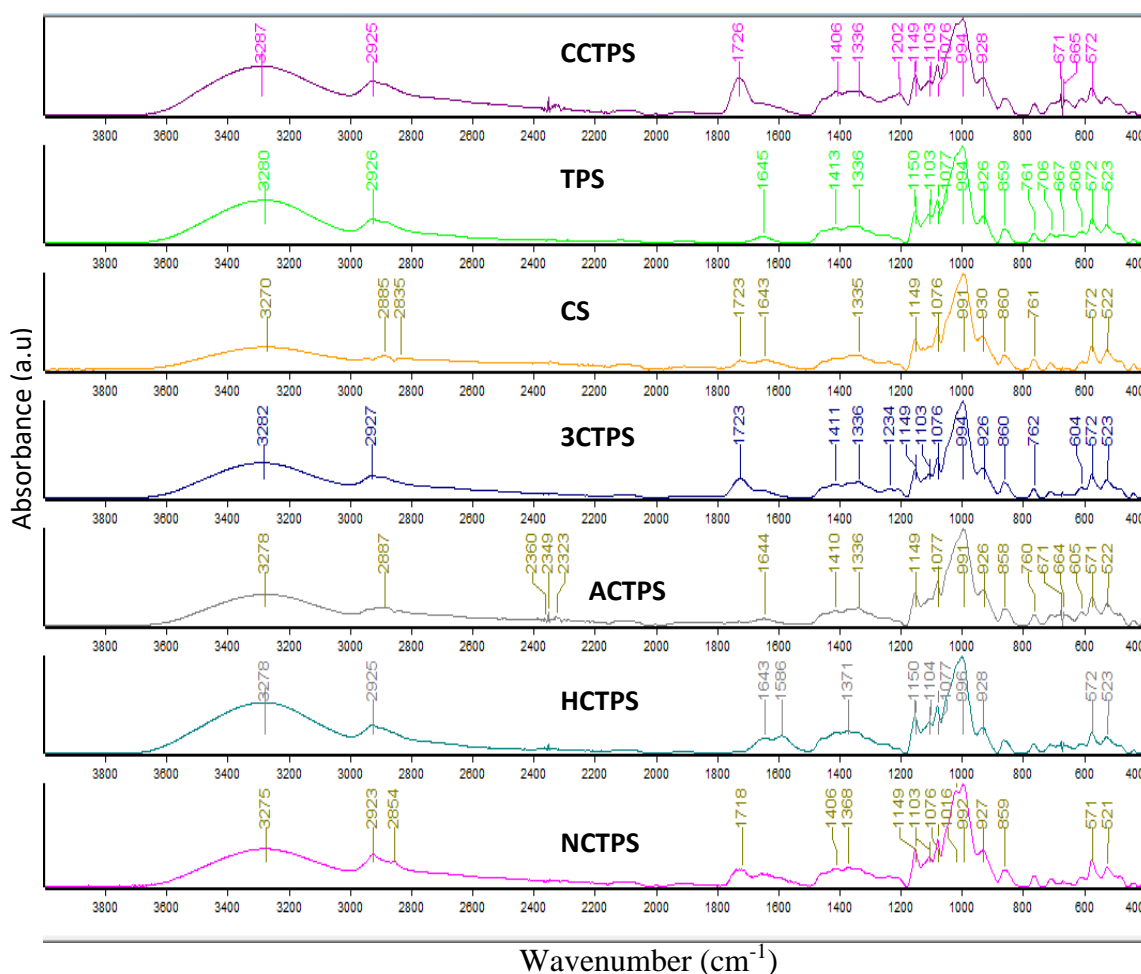


Figure 4. 1 Fourier Transform Infrared Spectroscopy Spectrum of the samples

The result of the FTIR test generally shows that TPS which is free of citric acid, ACTS (sample prepared by immersing TPS into a solution of citric acid and alcohol) and HCTPS (sample prepared at pH 12) are not crosslinked, which means the techniques followed don't favor crosslinking to happen. Samples CS (a sample prepared without glycerin as a plasticizer); and samples CCTPS, NCTPS, and 3CTPS prepared at a pH below 6 are crosslinked.

#### 4.1.2 Dynamic Mechanical Analysis

The Dynamic mechanical analyzer (DMA) is used to calculate the thermomechanical behavior of materials based on their thermomechanical responses loss factor ( $\tan \delta$ ) and storage modulus ( $E'$ ). Fig. 4.2, shows the dynamic mechanical analysis result of the prepared samples. The curves mainly represent the modulus (loss modulus and storage modulus) and  $\tan \delta$  (the ratio between the storage modulus and loss modulus). This test is mainly used in this research to determine the stiffness and viscoelastic property of the samples.  $\tan \delta$  curves show the internal friction of mechanical damping in the system. An increase in the value of  $\tan \delta$  value shows that the material is viscous, and a value low shows that the material is elastic.  $\tan \delta$  curve of the samples TPS and ACTPS have sharp picks each at  $-50\text{ }^{\circ}\text{C}$  and ( $-64\text{ }^{\circ}\text{C}$  and  $7\text{ }^{\circ}\text{C}$ ) respectively. The  $\tan \delta$  curve of the samples 3CTPS, NCTPS, HCTPS, and CCTPS shows single sharp picks at  $12\text{ }^{\circ}\text{C}$ ,  $12\text{ }^{\circ}\text{C}$ ,  $12\text{ }^{\circ}\text{C}$ , and  $3\text{ }^{\circ}\text{C}$  respectively. The sharp peak on the  $\tan \delta$  curve normally shows the glass transition temperature ( $T_g$ ) of the materials. The glass transition temperature of the crosslinked samples is shifted to a higher temperature due to the effect of crosslinking by citric acid than the non-cross linked TPS and ACTPS which were prepared in a different technique.

The  $\tan \delta$  curve shown in fig.4.2, generally is higher for 3CTPS, NCTPS, HCTPS, and CCTPS and lower for TPS and ACTPS. This also could be due to the cross linking of the samples by citric acid, which in turn has increased the viscosity [144].

Fig.4.2 also shows the comparison of storage modulus curves for all samples tested. The storage modulus of the samples TPS and ACTPS is less than the storage modulus of NCTPS, CCTPS and HCTPS. This shows that the crosslinked samples are stiffer than the non-crosslinked samples. This result is consistent with the finding of Simões, Bruno Matheus et.al. [144].

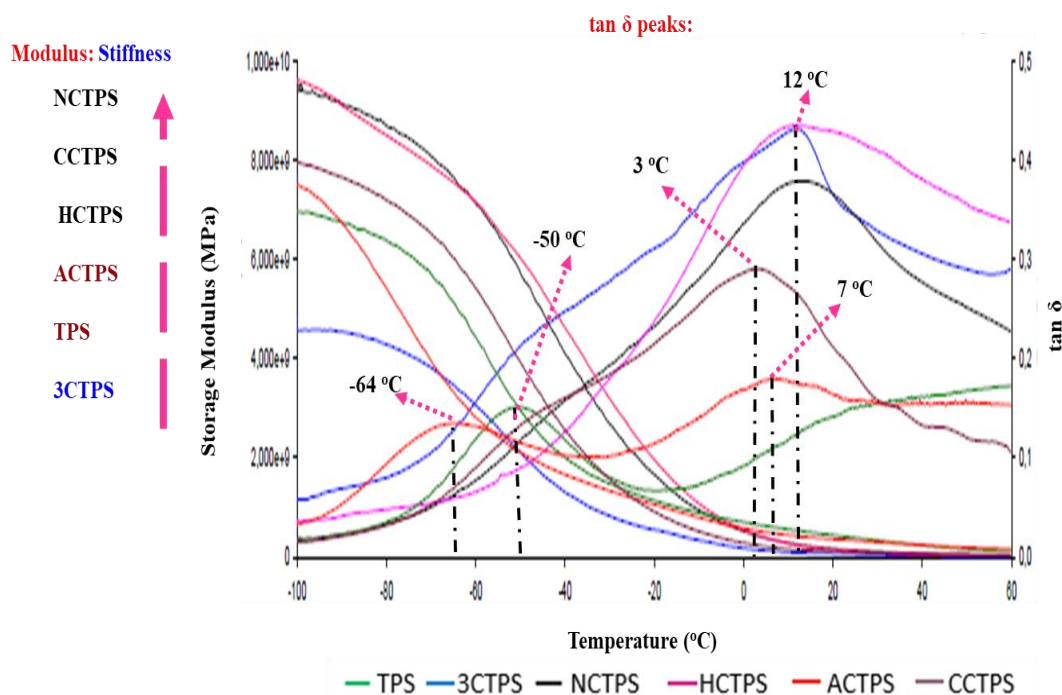


Figure 4. 2 Dynamic Mechanical Analyzer (DMA) Thermogram of Samples

#### 4.1.3 Surface Morphology

The morphology of the starch film samples prepared under different conditions and variable agents is studied by scanning electron microscope magnified 1000X and the results are presented in fig. 4.3. Samples TPS and CCTPS show similarity in their surface morphology that looks like an entangled polymer chain. This result indicates that the addition of glycerin to the samples has helped in plasticization and as a consequence long chains are formed. This also indicates that curing of starch films at 165 °C during preparation has a negative effect on crosslinking. The morphology of HCTPS shows agglomerations of NaCl gained from the reactions when NaOH and HCl are added to increase and decrease the pH of the system respectively. This has negatively affected the crosslinking performance and related properties. The morphology of ACTPS is also like TPS, but the solution of citric acid and alcohol which the sample was immersed does not allow a clear image of the surface of the starch film. The morphology of the starch film samples CS, 3CTPS, and NCTPS show a homogeneous and similar picture, showing the uniform dispersion and crosslinking of the starch granules. Crosslinking has modified the size and aggregation of the starch granules.



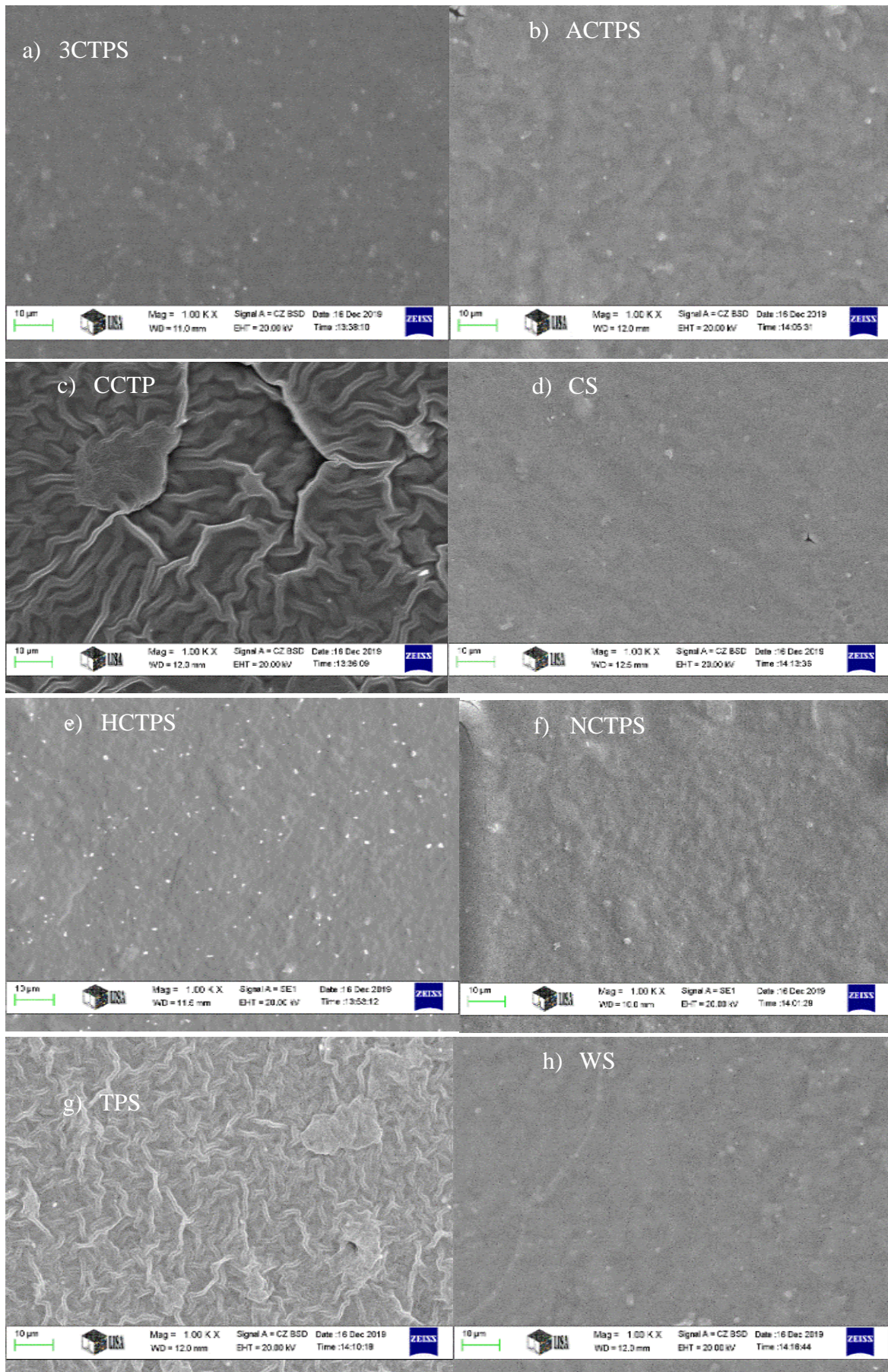


Figure 4.3 SEM Scans of Samples

#### 4.1.4 Thermal Analysis Using Differential Scanning Calorimetry

The heat effects of phase transitions and chemical reactions as they change with temperature are measured using differential scanning calorimetry (DSC). It measures the heat flow between a sample and a standard.

Three cycles are commonly used in DSC measurements for polymers (particularly thermoplastics). Heating, controlled cooling, and then heating again, i.e., the first heating cycle, cooling, and second heating cycle. The first heating scan is primarily used to remove residual solvents and erase the polymer's thermal history. The sample is then given a new thermal history during controlled cooling, with the cooling rate influencing the possible crystallization processes.

Information about the sample's new controlled thermal history is obtained during the second heating. Therefore, for most conventional polymers, the second heating cycle is preferably considered for thermal analysis. However, there are some exceptions, particularly for biopolymers such as starch and proteins, where the major transitions occur in the first run, such as starch gelatinization or protein denaturation.

Fig. 4.4 shows the DSC thermogram of the films (samples) prepared by mixing citric acid as a crosslinking agent and the free films without citric acid. All the sample films have an endothermic peak at about 120 °C both on the first and second heating cycles which is against the finding by Reddy et. al. [38] which states a shift of the endothermic peaks of the cross-linked films to a higher temperature when compared with non-cross-linked films in the DSC thermogram. The first endothermic peaks exist in both heat cycles, but are narrow in the second heating cycle. Therefore, the first endothermic peaks on the first heating cycle could be due the evaporation of moisture existing in the starch polymer film and the crystal relaxation/ chemical decomposition of starch molecules. The first endothermic peaks on the second heating cycle could be due to the crystal relaxation/ chemical decomposition of starch molecules, as the moisture is already evaporated in the first heating cycle. On the same DSC thermogram, TPS showed a second endothermic peak ( $T_m$ ) at about 170 °C, this could be due to the plasticization of the starch by glycerin which changed the semi crystal structure of starch into more amorphous structures with a lesser melting point. Except TPS, all the other starch film doesn't show a second endothermic peak ( $T_m$ ) at the specified range, the shifting of the second endothermic peak ( $T_m$ ) to a higher level on the starch film samples indicates that the inclusion of citric acid as a cross-linking and/or as impurity has affected to the increase in the melting point of starch films.



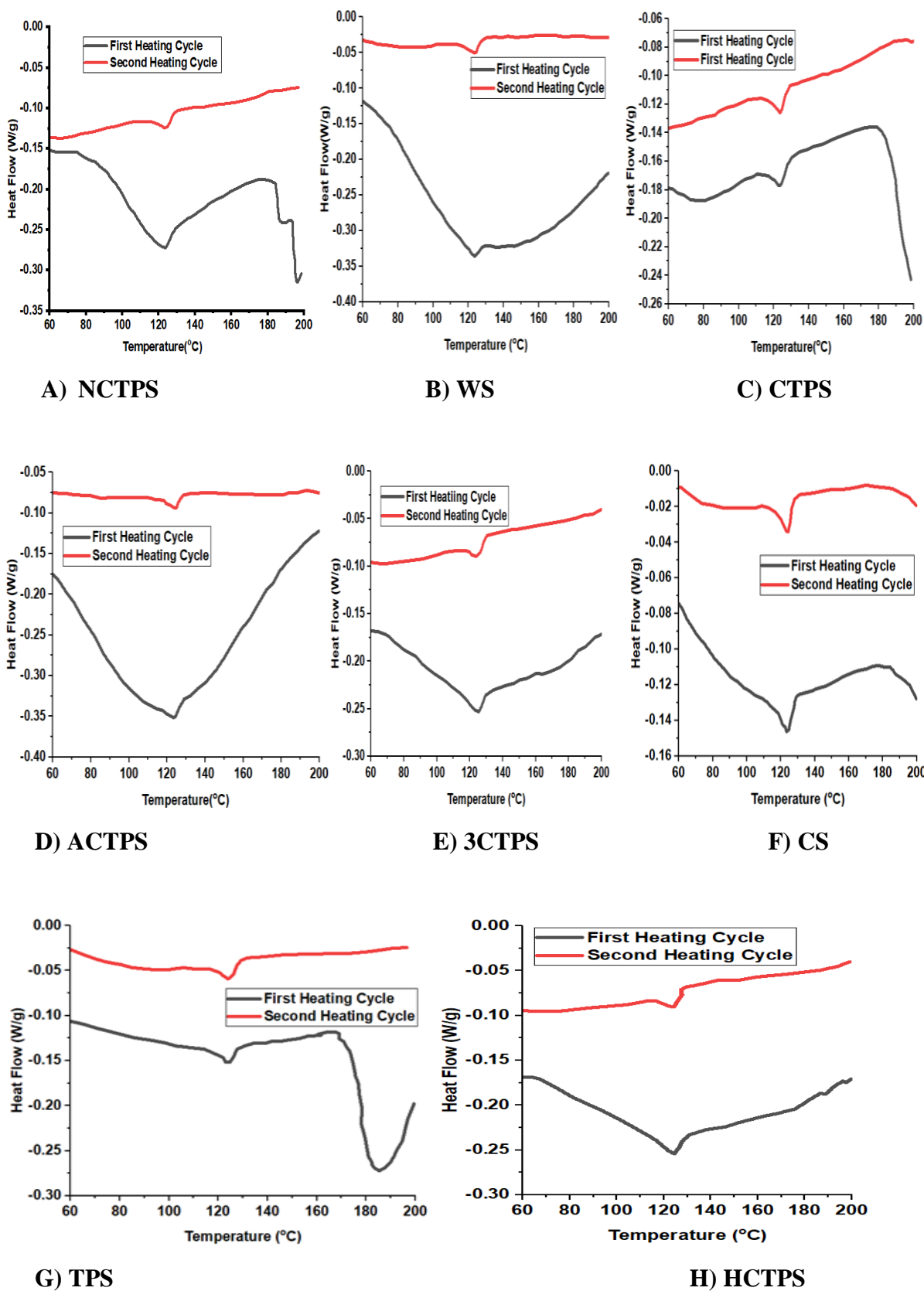


Figure 4. 4 DSC thermogram of samples

#### 4.1.5 Water Absorption Properties

Native starch is hydrophilic and crosslinking of the starch chains after plasticization by citric acid is expected to reduce the water absorption rate. Fig.4.1 shows that all the samples have high absorption rate in the first 20 minutes of wetting time. It is also evident in fig. 4.1 that the non-plasticized starch (WS) has the highest water absorption rate in the first 20 minutes and has started dissolving at about 20 minutes. This indicates that the plasticization of starch with glycerin has a positive effect on reducing the hydrophilicity of non-modified starch. 3CTPS (citric acid, starch film sample produced at a lower pH 3), NCTPS (non-cured; citric acid, starch film sample produced at about 6 pH) has got lower water absorption rate when compared with the CCTPS (CTPS cured at 165 °C for 10 minutes.). This shows that curing of the samples has negatively affected the water absorption property of the starch film. Curing might have created cracks or pores on the sample. The three samples prepared adding citric acid as a cross-linker; CCTPS, NCTS, and 3CTPS has lower water absorption rate and started dissolving later than TPS, WS, HCTPS and ACTPS. This is an evidence that esterification reaction has occurred, and this reaction has reduced free hydroxyl groups which in turn reduced the hydrophilicity. The results have confirmed what was reported in the literature [145]–[147]. The technique of crosslinking the surface of the starch film which was used to produce sample ACTPS doesn't alter the hydrophilicity of the starch.

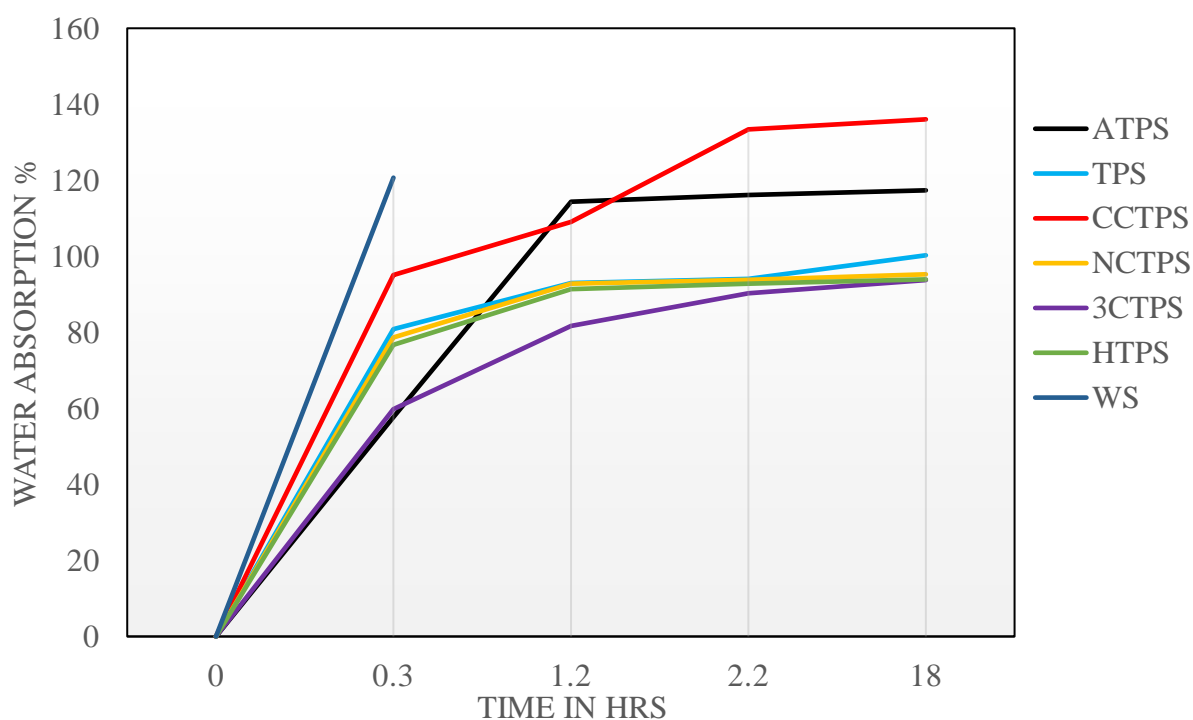


Figure 4. 5 Water Absorption Test Result

#### 4.1.6 Summary

Starch film samples prepared using different techniques and reagents were characterized for their water absorption, surface morphology, thermal and mechanical property, and structural change. Samples 3CTPS, NCTPS and HCTPS are prepared at 3, 6, and 12 pH respectively, to assess the effect of pH on crosslinking potential. Crosslinking was found better on NCTPS and 3CTPS, but lower on HCTPS. This shows that crosslinking/esterification reaction among citric acid, glycerin and starch is better achieved at lower pH and much better when done at about 6 pH. Samples NCTPS and CCTPS were prepared to evaluate the effect of curing on crosslinking potential. CCTPS is sample NCTPS cured at 165 °C for 10 minutes. Result has indicated that NCTPS is better crosslinked than CCTPS; which is against most of the reports done on this area, even if some reports have also supported this finding. ACTPS was prepared in a new and innovative method to crosslink the surface of the TPS film immersing it into a solution of citric acid and alcohol at about 70 °C; but currently results are showing no significant change in properties compared to the controlled TPS sample. CTPS, and CS are prepared to assess the effect of glycerin on the degree of crosslinking. Results showed that the availability of glycerin is a critical factor for the crosslinking and plasticity property of the film. CS samples were brittle and easily disintegrated in water as crystals. Generally, citric acid has increased the water resistance of starch crosslinking the chains of starch molecules and reducing the amount of hydroxyl group.

## **4.2 A Comparative Study of Carboxylic Acids on the Cross-Linking Potential of Corn Starch Films**

This experimental work is intended to produce starch film samples cross-linked with tartaric, oxalic, and citric acid and correlate the structure and property of carboxylic acids to the cross-linking potential and property of starch films used for non-food applications.

In this experiment, 8 g of native corn starch was added to 200 g distilled water. 2.88 g glycerol was then added to the slurry to prepare TPS, consequently, 1.6 g tartaric, citric and oxalic acids were added as crosslinking agents to prepare samples TaTPS, CTPS and OTPS respectively.

### **4.2.1 Characterization of the Thin Film Samples**

#### **4.2.1.1 Fourier Transform Infrared Spectroscopy**

The FTIR spectra of native (NST), gelatinized (TPS), and cross-linked starch using oxalic (OTPS), tartaric (TaTPS), and citric (CTPS) acids are shown in fig.4.6. The symmetric and asymmetric vibration of the C-H bond causes the band  $2929\text{ cm}^{-1}$ . The hydroxyl groups of starch are assigned the broad band  $3298\text{ cm}^{-1}$ . Peaks in the  $993\text{-}1263\text{ cm}^{-1}$  range are expected as a result of the stretching vibration of the C-O bond. Peaks at  $1664\text{-}1668\text{ cm}^{-1}$  are assigned to the C-O bending associated with the OH group in starch molecules and are present in all samples. The appearance of new peaks on the FTIR spectrum at  $1728\text{ cm}^{-1}$ ,  $1725\text{ cm}^{-1}$ , and  $1738\text{ cm}^{-1}$  for samples prepared with citric, oxalic, and tartaric acids, respectively, indicates that a reaction has occurred between the acids and starch. These peaks reveal C=O stretching vibration caused by ester formation as a result of cross-linking. The absence of these peaks in NST and TPS indicates that the cross-linking is primarily caused by the reaction of starch and acids. Subsequently, it can be stated that TaTPS, OTPS, and CTPS have the potential of cross-linking starch forming ester as a result of the cross-linking reaction.

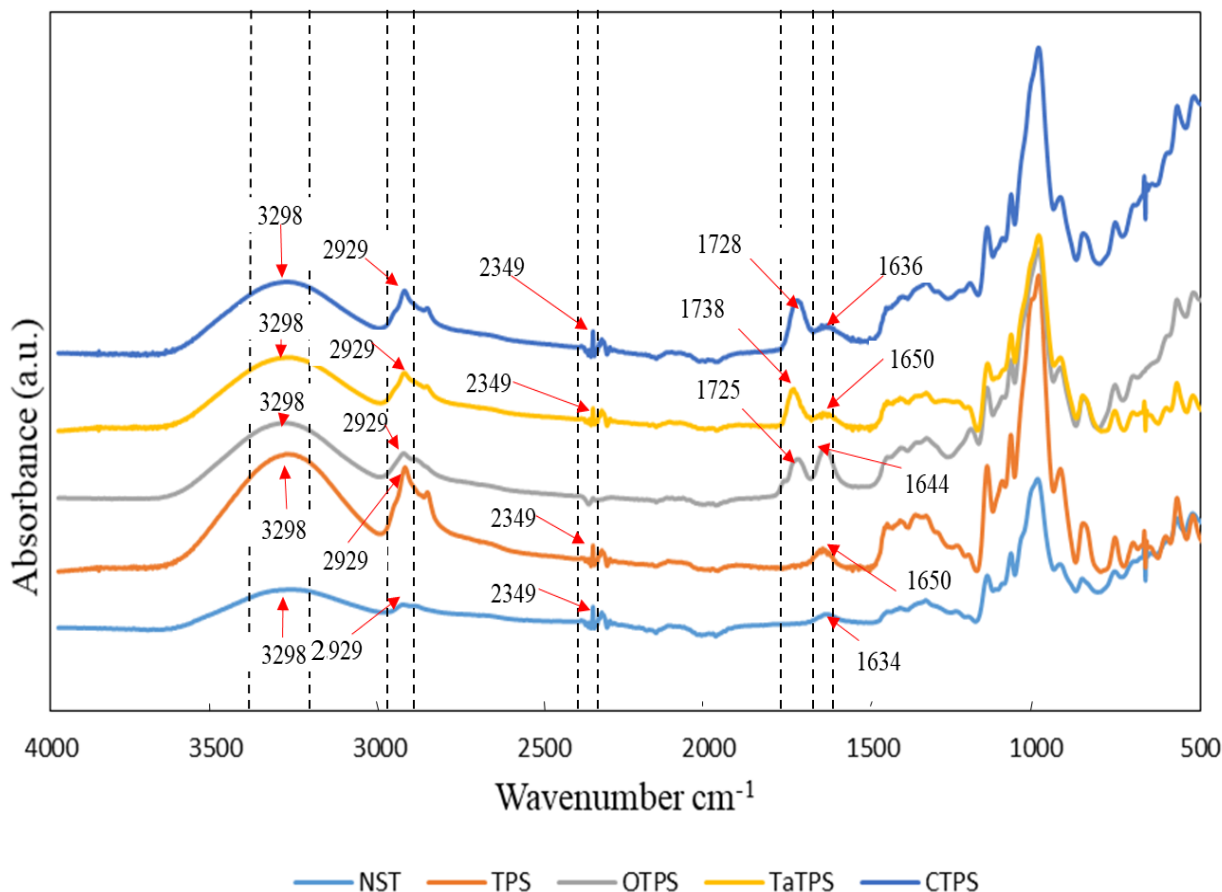


Figure 4. 6 Fourier Transform Infrared Spectroscopy Spectrum of Samples

#### 4.2.1.2 Surface Morphology

The morphology of starch film samples prepared with tartaric acid, oxalic acid, and citric acid as cross-linkers and glycerol as a plasticizer was examined using a scanning electron microscope. Fig. 4.7 depicts the surface morphology differences between TPS, TaTPS, OTPS, and CTPS, with TPS and CTPS exhibiting greater uniformity and homogeneity as a result of gelatinization and cross-linking, respectively. The uniformity of the surface morphology in TPS is due to the incorporation of water and glycerol into the starch granule during heating and continuous stirring, which has resulted in the loss of surface crystal stature. This is consistent with the finding that mixing glycerol, water, and starch for more than 15 minutes at a relatively high temperature; results in a homogeneous appearance with no significant presence of defects [148]. Similarly, using citric acid as a cross-linking agent effectively changed the crystal structure of starch into a uniform amorphous polymer, as shown in fig. 4.7, c, below. OTPS

and TaTPS samples that were cross-linked with oxalic and tartaric acid, on the other hand, had rough surfaces.

In the process of drying the samples at a temperature of 110 °C, crystals were observed to cover the surface of the oxalic acid cross-linked starch (figure 4.7,d), which may have been caused by the excess oxalic acid crystallizing [149].

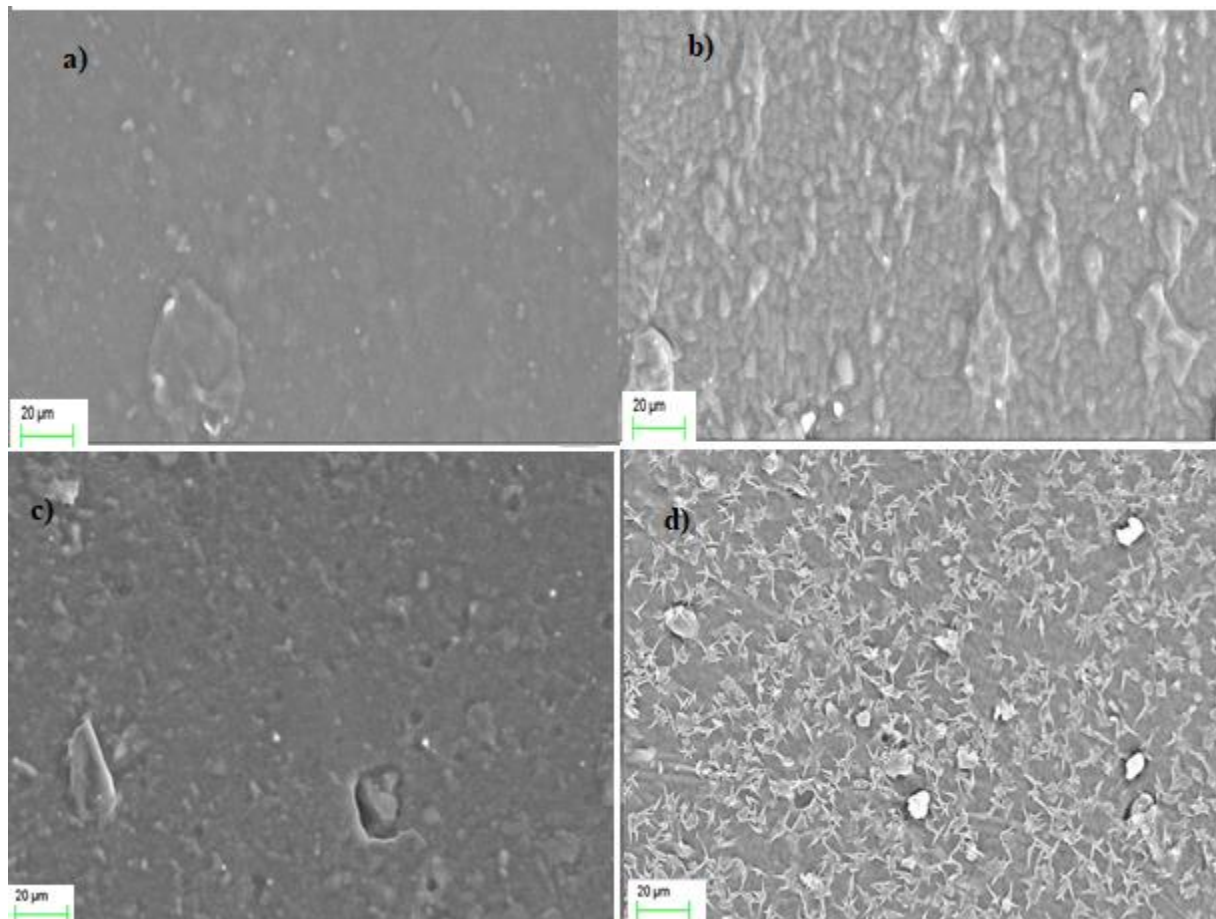


Figure 4. 7 SEM Scans of Samples a) TPS, b) TaTPS, c) CTPS, d) OTPS

#### 4.2.1.3 X-Ray Diffraction

Native Corn starch is a naturally semi-crystalline material made up of amylose and amylopectin with sharp diffraction peaks in the range of 15-24° (2 $\theta$ ) [150]. The angular diffraction pattern in figure 4.8 in the native corn starch (NST) shown below clearly indicates strong peaks at 15°, 17°, 18°, and 24° of 2 $\theta$  [151], which is the A type crystal structure polymorph of corn starch.

The intensity of the diffraction peaks decreases from native starch (NST) to gelatinized thermoplastic starch (TPS) to cross-linked once OTPS, TaTPS, and CTPS, as shown in the diffractograms figure 4.8. After being gelatinized to TPS with glycerol as a plasticizer, the diffraction peaks of NST are shifted to a single peak of 20°. After the esterification reaction

with oxalic and tartaric acid, the sharp peaks of NST are reduced to three peaks at 17°, 20°, and 22°. The diffraction pattern for the starch cross-linked with citric acid is almost flattened, indicating that the polymer is transitioning from crystalline to amorphous [85].

This generally indicates that citric acid has the highest degree of cross-linking, followed by tartaric acid and oxalic acid in that order. The esterification reaction destroys the crystalline nature of starch and forms long chains of polymers, decreasing the diffraction peaks in the modified starch [152]. This is because, during the cross-linking and gelatinization processes, the hydrogen bonds of starch are destroyed by glycerol and water, and acid enters the crystalline starch granules.

The cross-linked reaction by oxalic and tartaric acid, as well as the gelatinization process of starch, did not significantly change the crystallinity of starch when compared to the film cross-linked using citric acid. Because tartaric acids have more OH groups than citric acid, the hydroxide groups that remain in the starch molecules may have oxidized with hydrogen peroxide to form starch crystals and maintained the crystallinity of starch in the film. This is proved This is in agreement with the finding of Christian Seidel et al. [110]. The relative degree of crystallinity determined from the XRD diffractograms table 4.3 also proves that citric acid has reduced the crystallinity of native corn starch from 36.62% to 27.44%.

Table 4. 2 Relative degree of crystallinity of samples computed from the XRD graph

<b>Samples</b>	<b>NST</b>	<b>TPS</b>	<b>CTPS</b>	<b>TaTPS</b>	<b>OTPS</b>
Degree of Crystallinity (%)	36.62	31.43	27.44	29.63	30.92

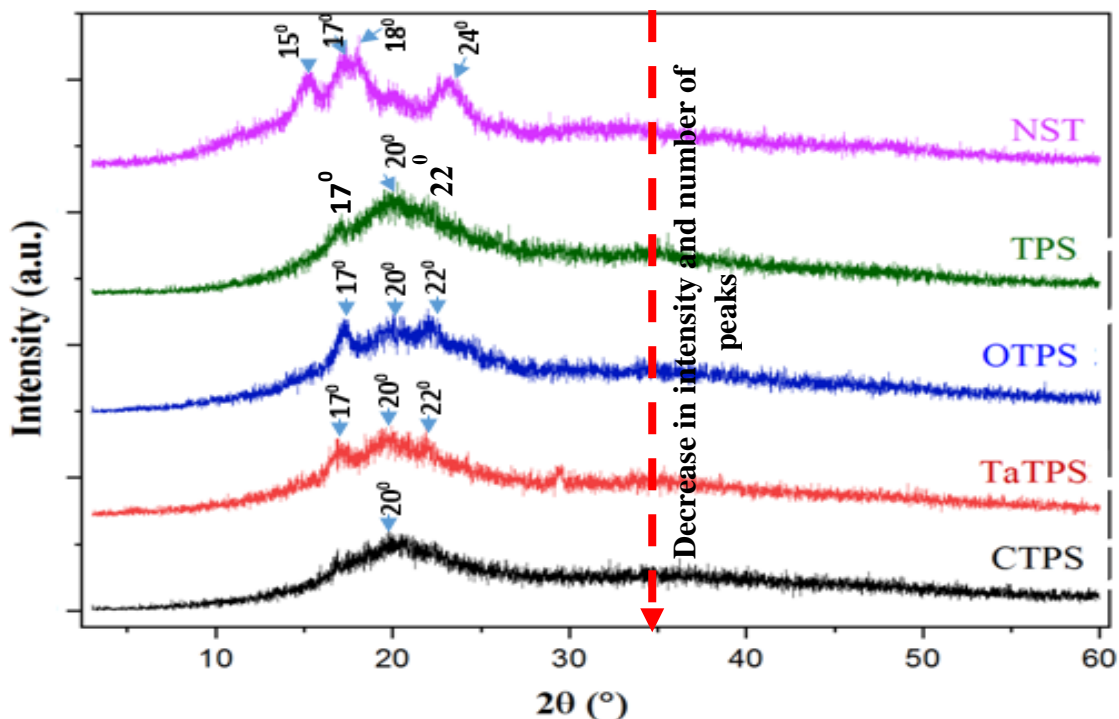


Figure 4. 8 X-Ray Diffractograms of Modified Starches

## 4.2.2 Thermo-Mechanical Properties of the Cross-Linked Thin Films

### 4.2.2.1 Thermal Properties of the Starch Films

Figure 4.9 shows the DSC thermogram of the films (samples) prepared by mixing citric acid, tartaric acid, and oxalic acid as a cross-linking agent and the non-cross-linked films without acid. Sample films TaTPS, TPS, and OTPS except CTPS have endothermic peaks at 130 °C, 120 °C, and 105 °C at the first heating cycle, respectively. On the other hand, CTPS has no endothermic peak on the first heating cycle. The endothermic peaks of all the samples disappeared on the second heating cycle. In the first heating cycle, the endothermic peaks are expected due to the evaporation of water from the sample. Thus, the shift of the endothermic peaks in the first heating cycle from 105 °C in OTPS to a higher temperature in TaTPS and to complete disappearance in CTPS might have been caused by the difference in the shape of the measured materials [153]. The shift in the endothermic peak to higher temperature after cross-linking agrees with the finding of Reddy et al. [154], which states a shift of the endothermic peaks of the cross-linked films to a higher temperature when compared with non-cross-linked films in the DSC thermogram but goes against the finding for the oxalic acid cross-linking starch [155].



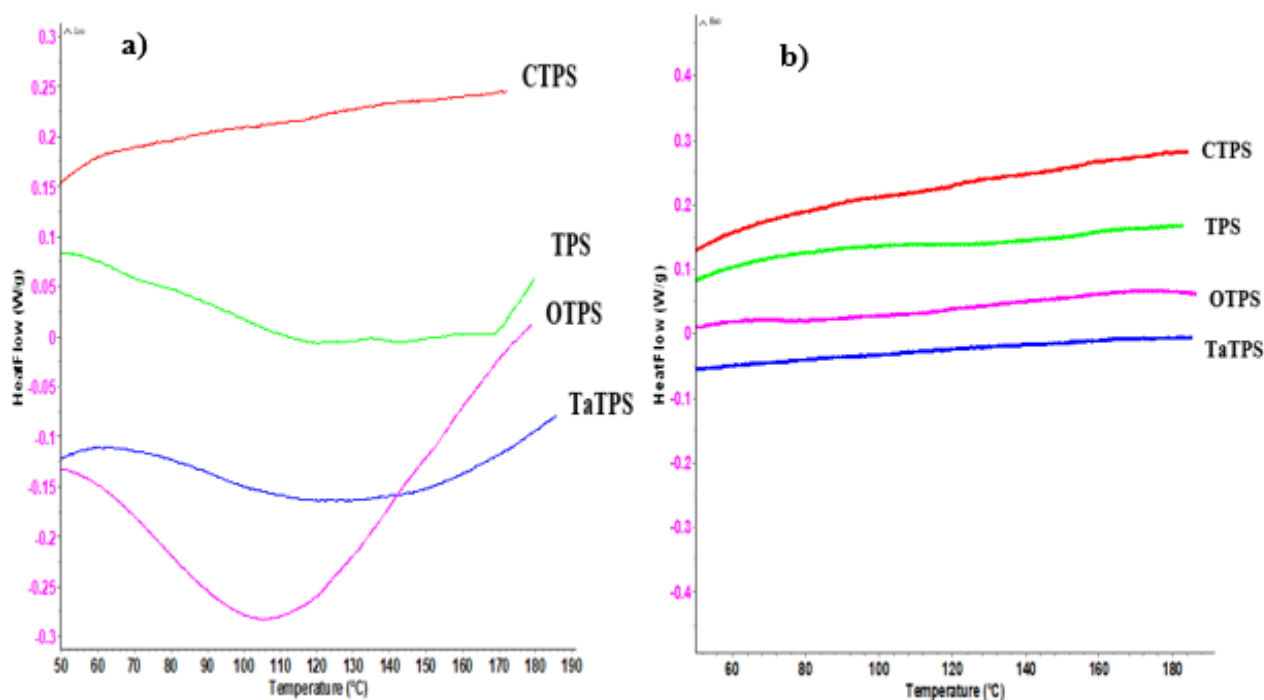


Figure 4. 9 DSC Thermograph of Cross-Linked and Gelatinized Starch Films a) First Heating Cycle b) Second Heating Cycle

#### 4.2.2.2 Dynamic Thermo-Mechanical Property

The dynamic mechanical analysis result of the prepared samples is shown in figure 4.10 below. The curves are generated by scanning the samples at four different frequencies: 0.1, 1, 3, and 10 hertz.  $\tan \delta$  peaks indicate the temperature at which the material's structure relaxes due to glass transition or melting.

Many authors have previously reported that glycerol gelatinized starch is a partially miscible system [156]–[158]. The  $\tan \delta$  curve in figure 4.10, a, which corresponds to TPS, has two peaks, indicating that the material goes through two relaxation phases below and above the ambient temperature; the first relaxation temperature is for glycerol-rich TPS, and the second relaxation temperature is for starch-rich TPS.

In contrast, cross-linked starch has only one peak, and if it exists, the first peak is very weak. This clearly shows that the cross-linking has restricted the first relaxation phase and limited the material to a single glass transition relaxation phase, consistent with previous findings [159]. The lower glass transition temperature of CTPS compared to TPS indicates that citric acid cross-linking of starch has effectively changed the material into an amorphous material with increased elasticity. Similarly, the decrease in  $\tan \delta$  value in the modified samples is primarily

due to acid cross-linking of starch. This means that the modified samples have a lower viscosity than TPS.

The storage modulus curve depicts the elastic behavior of materials over a temperature range and indicates the material's stiffness change. As shown in figure 4.10, the samples' storage modulus ( $E'$ ) decreases as the temperature rises from  $-130\text{ }^{\circ}\text{C}$  to  $100\text{ }^{\circ}\text{C}$ , except for oxalic acid cross-linked starch, which increases after  $40\text{ }^{\circ}\text{C}$  to  $80\text{ }^{\circ}\text{C}$  and then decreases to  $100\text{ }^{\circ}\text{C}$ . This indicates that Cross-linking reaction between oxalic acid and starch was happening during the DMA measurement [37].

Figure 4.10 a-d shows that the  $T_g - \tan \delta$  increases as frequency increases. This is because materials tend to behave more viscously and more like liquids when the frequency is low; and behave more elastically and more like solids when the frequency is high [160].

Logarithmic transformation of eq. (2) results;  $\ln(f) = \text{Activation Energy}/(RT)$ . The Arrhenius plot is constructed based on the maximum temperatures of  $T_g\delta$  on the loss factor curve. The slope of the Arrhenius plot is equated to activation energy/R, i.e., Slope =  $-E_a/R$ . Therefore, activation energy is found by multiplying the slope of the Arrhenius plot by the universal gas constant.

The results of the activation energy values calculated and the Arrhenius plot of each sample are clearly described in table 4.3 and figure 4.11 below, respectively. Less activation energy means that a small amount of energy is needed to displace and move the chains. Less activation energy of CTPS  $141.7\text{ J/mol}$  compared to TPS2 (the starch-rich TPS) and TaTPS (table 4.3) means that less energy is required to move and displace polymerized and esterified chains of starch. The high activation energy, high glass transition temperature, and low activation energy low glass transition temperature correlation are validated from the results. Calculating the activation energy of OTPS was impossible because the peaks on the curve do not represent the relaxation temperature values but rather to cross-linking reaction and/or evaporation of excess water during measurement.

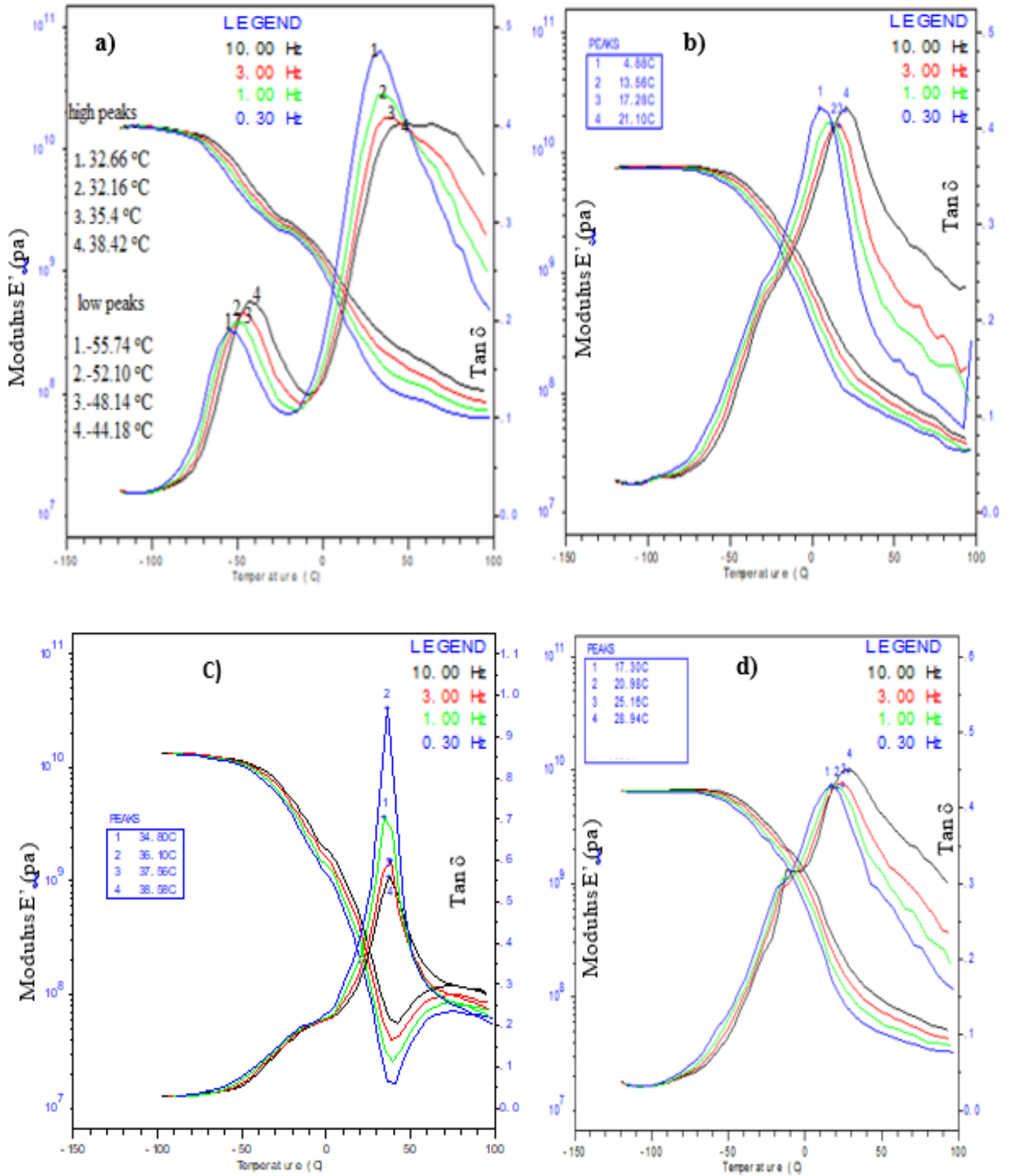


Figure 4. 10 Dynamic Mechanical Analyzer Thermograms of Samples a) TPS b) CTPS c) OTPS d) TaTPS

Table 4. 3 Low and high peak glass transition temperature of samples and the calculated activation energy

Samples		TPS1	TPS2	CTPS	TaTPS	OTPS
F (HZ)	ln(F)	Tg δ (°C)	Tg δ (°C)	Tg δ (°C)	Tg δ (°C)	Tg δ (°C)
0.3	-1.20397	-55.74	32.66	4.88	17.27	34.8
1	0	-52.1	32.16	13.56	20.85	36.1
3	1.098612	-48.14	35.4	17.28	24.82	37.56
10	2.302585	-44.18	38.42	21.1	28.87	38.58
<b>Ea tan δ J/mol</b>		<b>124.4</b>	<b>376.6</b>	<b>141.3</b>	<b>218</b>	Impossible
r		0.9993	0.8379	0.9461	0.9991	

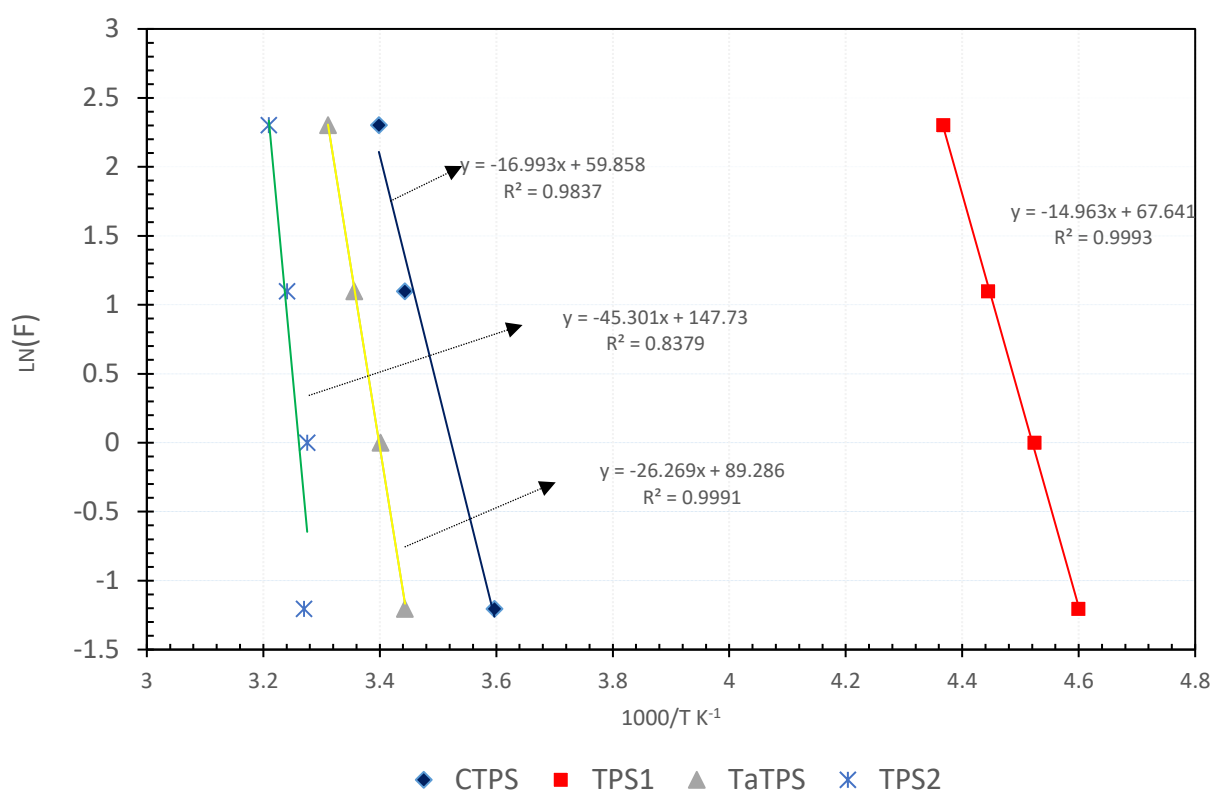


Figure 4. 11 Arrhenius plot constructed based on the maximum temperatures Tgδ (loss factor) on four different frequencies; high peaks of TPS (TPS2), low peaks of TPS (TPS1), and high peaks of TaTPS, high peaks of CTPS.

#### 4.2.2.3 Stress-Strain Property

Table 4.4 displays the tensile stress-strain test results of the gelatinized and cross-linked samples. The tensile strength of the crosslinked samples is higher than the non-crosslinked TPS (table 4.4), this is because cross-linking enhances the intermolecular interaction between starch molecules by forming ester bonds that interconnect the starch molecules [161]. Even though the number of hydroxyl groups in CA and TA is the same, TaTPS is less strong than CTPS due

to the higher number of carboxylic groups in CA than in TA. This could be because carboxyl groups can form stronger ester bonds with the hydroxyl groups of starch; than the hydrogen bonds that the hydroxyl groups can [162]. On the other hand, OA and TA have an equal number of carboxyl groups and differ in the number of hydroxyl groups. Therefore the increase in tensile strength of TaTPS over OTPS is due to the increased number of intra and intermolecular hydrogen bonds among starch molecules.

The elongation at break of CTPS is maximum followed by TPS, and TaTPS respectively. The increase in strain in CTPS films compared to TaTPS and OTPS films without affecting tensile strength could be due to the act of citric acid being used as a cross-linker and plasticizer changing the starch films from ductile to plastic properties [163]. The residual CA used as plasticizer has the capability to reduce the interaction between the macromolecules so that entangled molecules can have a relative freedom to freely stretch [112]. This could be another reason for the increase in elongation at break of CTPS. The extreme decrease in the tensile strain of oxalic acid cross-linked starch could be due to the less plasticization potential of residual oxalic acid compared to citric acid [90]. This is in agreement with the DMA test result shown in fig. 4.10, C. The additional carboxyl functional group in citric acid could have helped in disturbing starch's inter and intramolecular bonds to effectively plasticize starch film and amorphous molecules.

Table 4. 4 The Tensile Test Result of the Samples

<b>Samples</b>	<b>Maximum Tensile Stress</b> (MPa)	<b>Maximum Tensile Strain</b> (%)
TPS	2.39 ± 1.3	108.6 ± 7.6
OTPS	3.05 ± 0.9	58.5 ± 6.5
TaTPS	3.36 ± 1.2	89.3 ± 9.3
CTPS	3.95 ± 0.9	112.6 ± 11.1

### 4.2.3 Water Absorption Property

Starch's water resistance has been improved by cross-linking it with various agents. Although cross-linking improves starch water resistance, the type of cross-linking agent used has a significant impact. The water absorption curves Fig.4.12 show three distinct zones. The rate of absorption is very quick in the first twenty minutes of the first insertion; beyond that, the rate of absorption are slow and lead to a plateau, corresponding to the equilibrium of water absorption.

As a result of starch, glycerine, or acid dissolving back into the distilled water, the absorption curve curves down in the third zone. TPS, non-cross-linked thermoplastic starch, absorbs the most water compared to the other samples. The reduction in the curve for TPS after 18 hours of wetting is due to the sample dissolving in water. Glycerol and starch molecules absorb and dissolve in the distilled water, making the remaining water viscous.

The starch that was treated with oxalic acid completely disintegrates into fractions after wetting for the first two hours. Measuring the weight of the wetted sample became impossible after that, and the measurement was halted. The water absorption rate and dimensional stability of tartaric and citric acid cross-linked thermoplastic starch are comparable until 120 hours of wetting time. However, after 120 hours of wetting, tartaric acid cross-linked starch began slowly dissolving and was entirely destroyed. The citric acid-cross-linked starch retained its dimensional stability even after a wetting time of 168 hours. This result confirms the effectiveness of cross-linking in improving the water sensitivity of starch. Cross-linking TPS with citric acid significantly improved the water sensitivity of starch, followed by tartaric acid [164]. In contrast, using oxalic acid as a cross-linker adversely affects the water resistance of starch.

After hours of wetting, the colors of TPS and TaTPS gradually changed to white and yellowish, respectively. Yellow spots were clearly observed on the surface of the TaTPS sample. This may be due to the biodegradation of starch. TPS showed no colored spots on the surface, except for a gradual color change that could be due to the dissolution of glycerol from the sample into water. Unlike the other samples, CTPS retained its color and dimensional stability. Comparing the water absorption rate of TPS, which is about 140%, with the water absorption rate of CTPS and TaTPS, which is about 60%, as shown in fig. 4.12, it is concluded that there is a significant improvement in the water resistance of starch by cross-linking using citric acid tartaric acid.

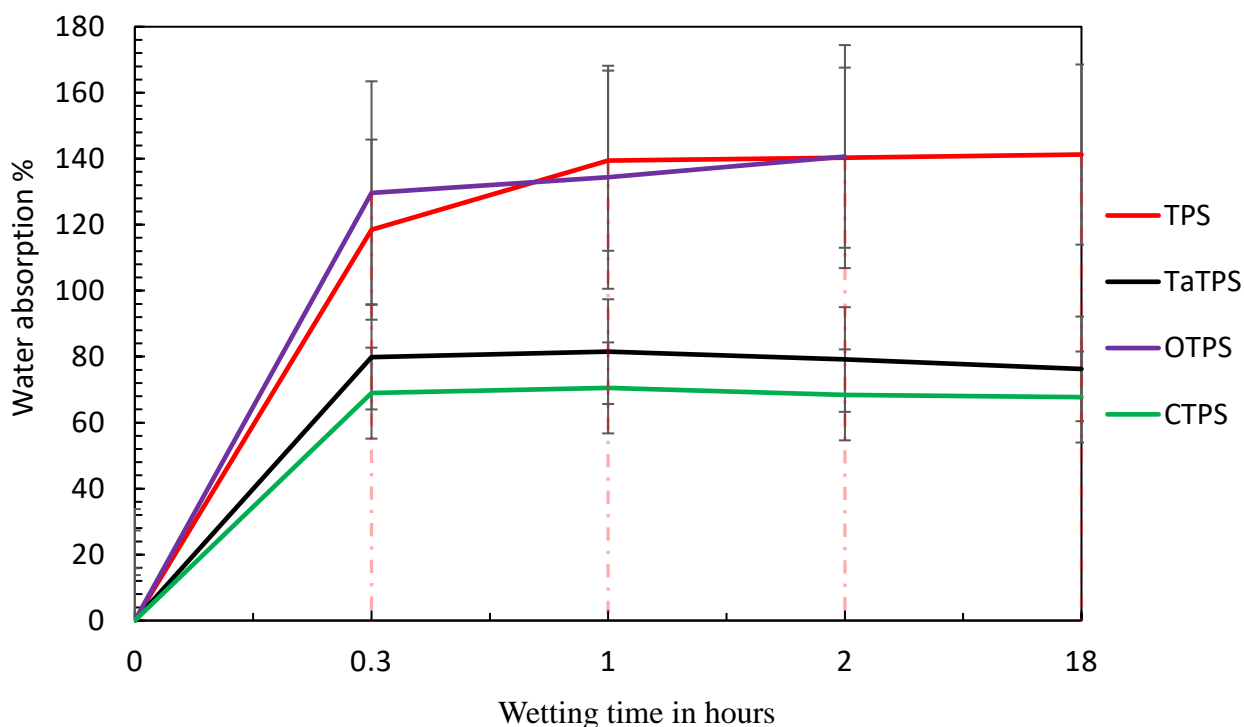


Figure 4. 12 Water Absorption Test Results

#### 4.2.4 Summary

Starch film samples were prepared using tartaric acid, citric acid, and oxalic acid cross-linking agents; and Glycerol and water as plasticizers. Samples were characterized and tested for water absorption, surface morphology, mechanical and thermo-mechanical properties, and structural change. FTIR test results revealed that citric, tartaric, and oxalic acids have cross-linked starch. The x-ray analysis exhibited a decrease in the diffraction peaks in the modified starch due to the esterification reaction that destroys the crystalline nature of starch and forms an entangled chain of amorphous structure. This could be due to the cross-linking and gelatinization process that destructs the hydrogen bonds of starch using glycerol, water, and the acid entering the crystalline starch granules. Citric acid cross-linked starch has significantly improved the strain (112.66 %) and tensile strength (3.95 MPa) of starch compared to TPS, TaTPS, and OTPS. Citric acid cross-linking has also improved starch's water resistivity and dimensional stability better than tartaric and oxalic acid. This could be due to increased carboxyl functional groups in citric acid than oxalic acid and tartaric acid. The improved property of TaTPS over OTPS is also due to the increased number of hydroxyl groups in tartaric acid than oxalic acid.

### 4.3 Effects of Acidity, Number of Hydroxyl Group, and Carbon Chain Length of Carboxylic Acids on Starch Cross-Linking

This experimental work is envisioned to produce starch film samples cross-linked with malic, malonic and succinic acids, and evaluate the effects of acidity, number of hydroxyl group, and carbon chain length on starch cross-linking.

In this experiment, 8 g of native corn starch was added to 200 g distilled water. And then 2.88 g glycerol was added to the slurry to prepare TPS; consequently, 1.6 g succinic, malonic and malic acids were added as crosslinking agents to prepare samples STPS, MoTPS and MaTPS respectively.

#### 4.3.1 Molecular Structure, and Surface Morphology

Fig. 4.13 shows the FTIR spectra of TPS, MaTPS, MoTPS, and STPS samples. In contrast to TPS, the appearance of new peaks on the FTIR spectrum at  $1717\text{ cm}^{-1}$ ,  $1712\text{ cm}^{-1}$ , and  $1718\text{ cm}^{-1}$  for samples cross-linked with malic, succinic, and malonic acids, respectively, indicates a reaction between the acids and starch. Peaks  $1717\text{ cm}^{-1}$ ,  $1712\text{ cm}^{-1}$ , and  $1718\text{ cm}^{-1}$  show C=O stretching vibration [165], [166] caused by ester formation as a result of cross-linking. The absence of such peaks in the TPS sample indicates that the cross-linking is primarily caused by the reaction between starch and the crosslinking acids [167]. Because of an increase in the number of ester bonds in malic and malonic acid cross-linked starch, the normalized intensity of the spectral peaks is seen to be higher than in succinic acid cross-linked starch and non-crosslinked TPS. When reacted with succinic acid, the less water solubility of succinic acid may have played a significant role in the reduction of ester bonds in the starch structure.

The band  $2923\text{ cm}^{-1}$  is due to the symmetric and asymmetric vibration of the C-H bond. The broadband  $3285\text{ cm}^{-1}$  is assigned to the hydroxyl groups of starch [148]. Peaks in the  $993\text{-}1263\text{ cm}^{-1}$  range are caused by the stretching vibration of the C-O bond [168]. The peaks at  $1664\text{-}1668\text{ cm}^{-1}$  are due to the C-O bending associated with the OH group in starch molecules and are present in all samples. According to the FTIR spectra, malic, malonic, and succinic acids have the ability to cross-link starch by forming ester bonds as a result of the cross-linking, with malic, malonic, and succinic acids ranking first, second, and third in efficiency, respectively.



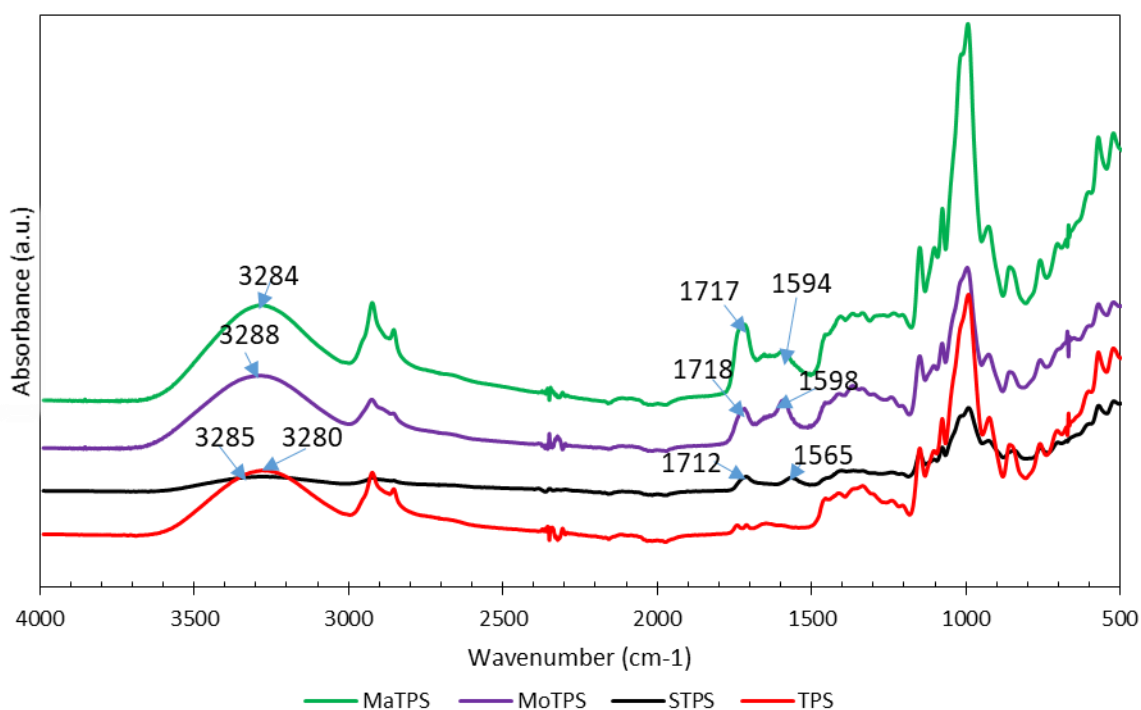


Figure 4. 13 Fourier Transform Infrared Spectroscopy (FTIR) Spectrum of samples

The morphology of the starch film samples prepared by adding malic acid, malonic acid, and succinic acid as a cross-linker and glycerol as a plasticizer are studied by Scanning electron microscope (SEM), and the results are presented in Fig. 4.14. Comparing the surface morphology of TPS, MaTPS, STPS, and MoTPS; TPS and STPS show relatively smooth granule structure on their surface. The appearance of some large in size, oval in shape and dispersed agglomerated granule layers in TPS (see red-colored circles), is derived from the semi-crystalline nature of native starch and the process of agglomeration during gelatinization [155]. As starch is a semi-crystalline material, it is observed that it keeps its semi-crystal nature in the gelatinized TPS sample [148]. On the contrary, the use of malic and malonic acid as a cross-linking agent has changed the surface of starch into an entangled long chainlike amorphous granule structure (see red dots) in Fig. 4.14 below. MaTPS and MoTPS samples which are cross-linked with malic and malonic acid, have shown entangled polymer starch granule surfaces as a result of cross-linking.

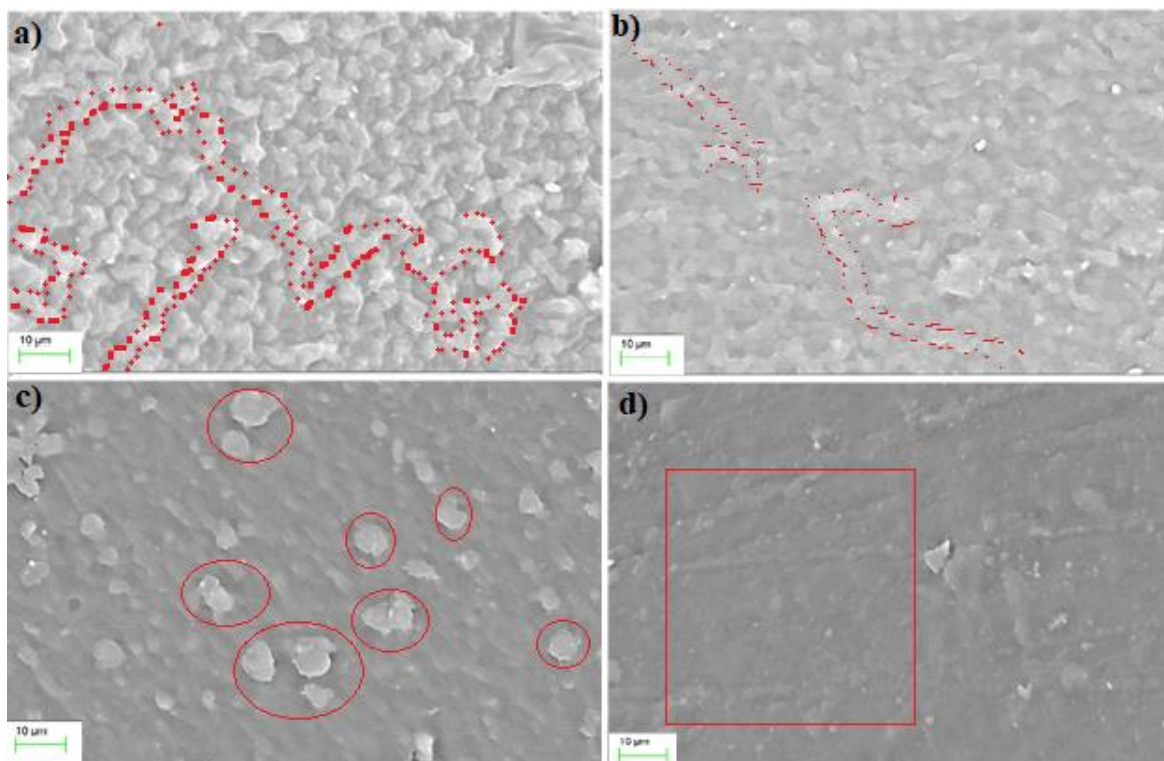


Figure 4. 14 SEM scans of samples a) MaTPS b) MoTPS c) TPS d) STPS

The intensity of the diffraction peaks decreases from TPS down to MoTPS, STPS, and MaTPS, as shown in the diffractograms in fig. 4.15. TPS diffraction peaks are found to be similar to native starch diffraction peaks, with some shifts and lower intensities. After the esterification reaction with malonic acid, the sharp peaks of TPS are reduced to three peaks at  $17^\circ$ ,  $20^\circ$ , and  $22^\circ$  but with less intensity than the single peak of succinic acid. The diffraction pattern for starch cross-linked with malic has changed into a single peak with very less intensity, indicating that the polymer has transitioned from semi-crystalline to a more amorphous structure [85]. This demonstrates that malic acid has a higher degree of cross-linking than malonic acid and succinic acid. The esterification reaction destroys the Semi-crystalline nature of starch and forms a long chain of polymers, resulting in a decrease in the diffraction peaks of starch in modified starch [152]. This is due to the hydrogen bonds of starch being deconstructed during the cross-linking and gelatinization processes by glycerol, water, and acid entering the crystalline starch granules.

The XRD graph depicts that, compared to the film cross-linked using malic acid, the cross-linked reaction by malonic acid and the gelatinization process of starch did not significantly change the crystallinity of starch. Because malonic acid has fewer OH groups than malic acid, the hydroxide groups that remained in the starch molecules could have oxidized with hydrogen peroxide to form starch crystals, preserving the crystallinity of starch in the film [169].

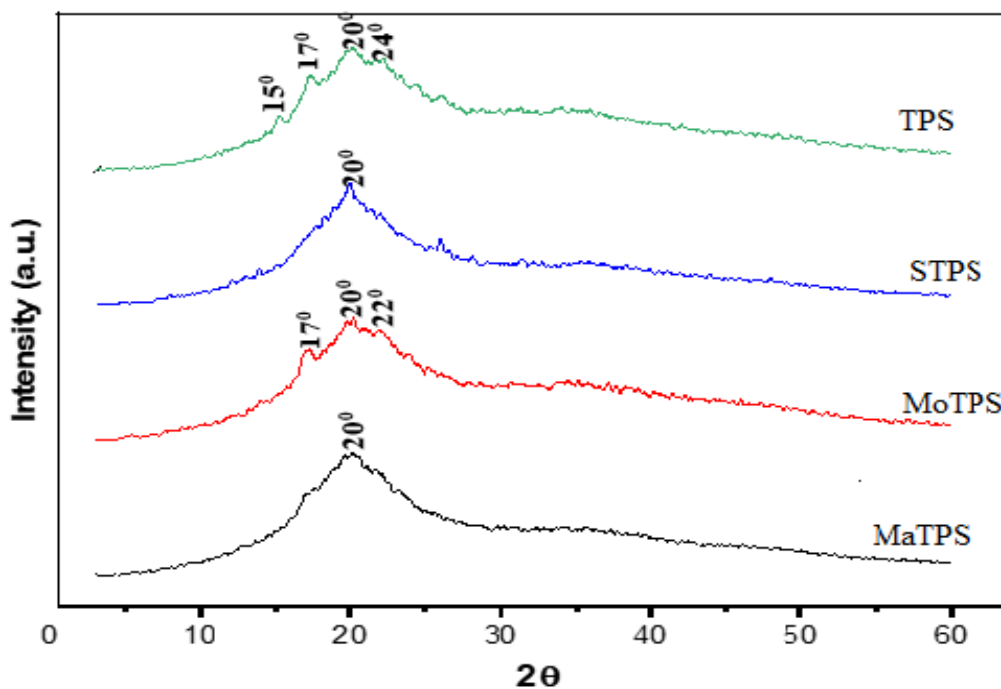


Figure 4.15 X-Ray Diffractograms of Modified Starches

#### 4.3.2 Thermal Properties

The DSC thermogram of the films (samples) MaTPS, MoTPS, STPS, and TPS are shown in fig.18. Except for TPS and STPS, samples MaTPS and MoTPS, have endothermic peaks at 105 °C, 130 °C, respectively, during the first heating cycle. On the second heating cycle, all of the samples' endothermic peaks vanished. Endothermic peaks in the first heating cycle are the result of water evaporation from the sample and possible cross-linking reactions during the experiment. The transition from endothermic peaks in the first heating cycle to complete disappearance in the second heating cycle indicates an increase in the difficulty of evaporating water from the samples [153]. This indicates that thermal processes like controlled heating should be utilized during manufacturing of cross-linked starch polymers for possible applications. The shift in the endothermic peak after cross-linking agrees with the finding of Reddy et al. [154], which states a shift of the endothermic peaks of the cross-linked films to a higher temperature when compared with non-cross-linked films [155].

The endothermic peak in the MoTPS in the first heating cycle of the thermograph Fig.4.19 a) indicates that there was more water bounded with malonic acid cross-linked starch than the other samples and is connected with high water solubility nature of malonic acid than malic and succinic acid, which in turn is due to the decreased carbon chain length in malonic acid [170].

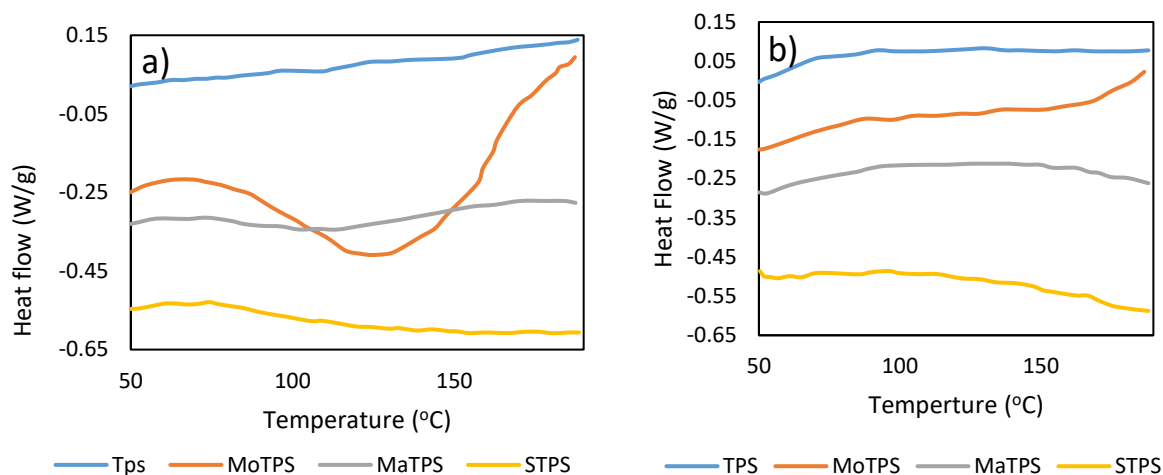


Figure 4. 16 DSC thermograph of cross-linked and gelatinized starch films a) First heating cycle b) Second heating cycle

### 4.3.3 Thermomechanical Behaviour

The dynamic mechanical analysis results of the prepared samples are shown in Fig. 17a.

The curves are generated by scanning the samples at a single frequency of 3 Hz. The peaks of  $\tan \delta$  tell the temperature at which relaxation on the structure of the materials occurs due to glass transition or melting.

TPS has two peaks in the  $\tan$  curve shown in fig. 4.17 a, indicating that the material goes through two relaxation phases below and above the ambient temperature; the first relaxation temperature is for glycerol-rich TPS, and the second relaxation temperature is for starch-rich TPS. In contrast, cross-linked starch samples have only one peak, and the second peak, if it exists at all, is very weak.

This demonstrates that cross-linking has restricted the first relaxation phase and limited the material to a single glass transition relaxation phase, which is consistent with previous findings [159]. The shift in the glass transition temperature of acid cross-linked starch to a lower temperature compared to TPS indicates that the cross-linking of starch using acid has changed the material into amorphous material with increased elasticity. The storage modulus curve shows the elastic behavior of materials under a range of temperatures and tells the change in the stiffness of the material. As is seen in fig. 4.17 b, the storage modulus ( $E'$ ) of the samples have decreased as the temperature increases from  $-120^{\circ}\text{C}$  to  $100^{\circ}\text{C}$ . The decrease is more noticeable at  $-40 - 100^{\circ}\text{C}$ , which is close to the glass transition temperature. The cross-linked starch samples showed sharper decrease in storage modulus than TPS. This could be due to chemical cross-linking and the formation of longer chains, which facilitated mobility [171].

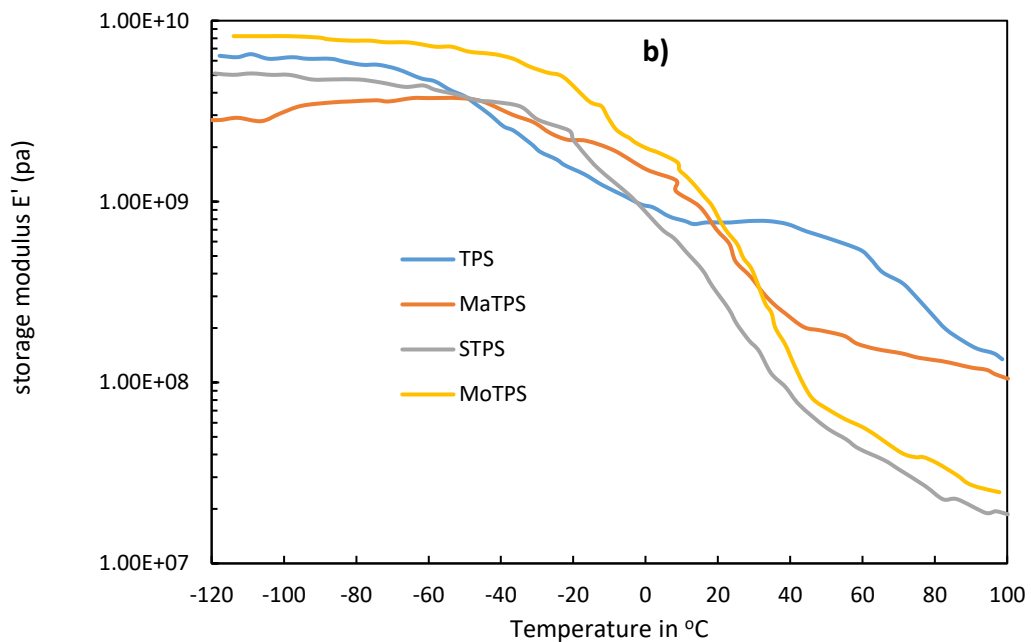
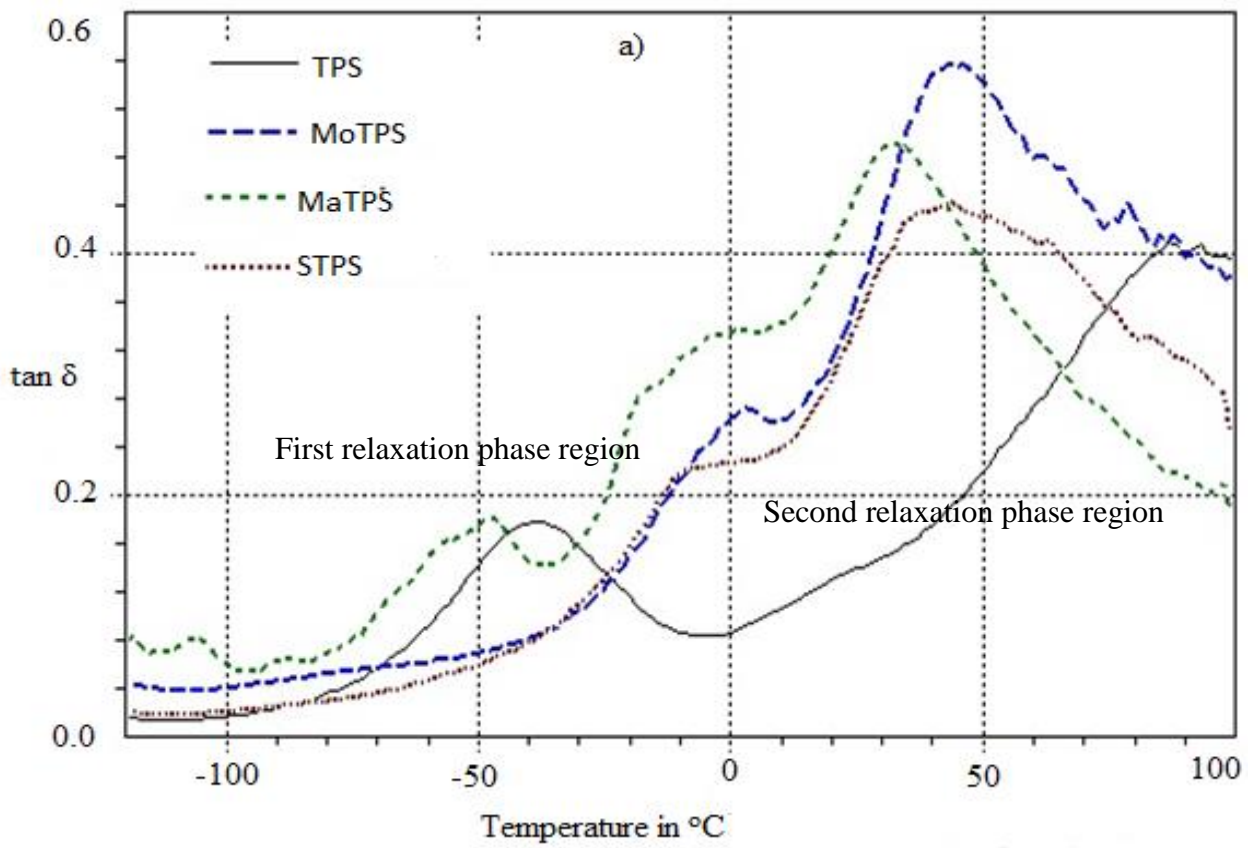


Figure 4. 17 Plots of DMA results: a)  $\tan \delta$  graph b) Storage modulus  $E'$  graph

#### 4.3.4 Mechanical Property (Stress-Strain Test)

Determining the tensile properties of starch films is necessary to confirm their suitability for future applications, such as food packaging. The molecular weight and amylose content of starch, film thickness, polymer chain packing, chain interaction, and crystallinity of the film can all influence these properties. Table 4.5 shows that the maximum tensile stress and maximum tensile strain of TPS are 2.39% and 108.63%, respectively, and that it has a relatively low tensile strain and tensile stress compared to the other samples. It could be because thermoplastic starch contains a higher concentration of hydrogen bonds and more crystalline structure. The maximum tensile strain, also known as elongation at break, of succinic, malonic, and malic acid cross-linked starch was measured to be 119.9%, 140.9%, and 175.7%, respectively see table 4.5, which is a significant improvement over TPS. This result is consistent with the findings of other similar studies [172, 173]. The increase in tensile strain is due to the change of starch from semi-crystalline structure to amorphous structure due to cross-linking (ester linkage formation).

The addition of sodium hydroxide before the addition of acids actually helped the swelling of the starch to a higher degree. The swelling happens due to breakdown of the intermolecular hydrogen bonds and the formation of new hydrogen bonds with water to form a larger, open structure. Alkali helps the de-bonding process by chain scissoring and helps the formation of new bonds within adjacent chains at the same time. This type of bond formation could help to improve the tensile properties of the materials. On the other hand, the addition of acids helps the breaking down of the bonds during swelling and more often results in better uniformity over the gelatinized paste. It may have helped improve the tensile strain properties of the starch. The cross-linked samples' tensile strength has increased compared to TPS, which is directly related to the transition of starch granules from semi-crystalline to an entangled amorphous structure with increased number of crosslinking bridges.

Succinic acid has the lowest tensile strength and young's modulus than malonic acid and malic acid, possibly due to its longer carbon chain length and fewer hydroxyl groups [172]. The mechanical properties of cross-linked starch obtained in this study were comparable to the mechanical properties of conventional polymers currently available on the market for packaging purposes, such as LDPE [174].

Table 4. 5 Tensile Test Results of Samples

<b>Samples</b>	<b>Modulus (Automatic Young's) (MPa)</b>	<b>Maximum Tensile Stress (MPa)</b>	<b>Maximum Tensile Strain (%)</b>
<b>TPS</b>	10.8 ± 6.9	2.4 ± 1.3	108.6 ± 7.7
<b>MoTPS</b>	50.7 ± 14.9	7.4 ± 1.4	140.9 ± 8.8
<b>MaTPS</b>	16.1 ± 2.	4.8 ± 0.5	175.7 ± 9.5
<b>STPS</b>	18.0± 2.3	2.4 ± 0.2	119.9 ± 5.2

### 4.3.5 Water Absorption Behaviour

Because of the large number of hydroxyl groups on its backbones, starch is extremely hydrophilic in nature. One of the major barriers to the use of starch-based products is their high water absorption. Water absorption has been shown to be strongly dependent on a variety of factors, including immersion time, interfacial adhesion, and the number of voids formed in the interfacial area etc [175]. As can be seen in fig. 4.18, all of the samples have a high absorption rate during the first one-third of an hour of wetting time. TPS absorbs water at a comparable rate to the sample cross-linked with malonic acid. Succinic acid cross-linked starch was discovered to be the most sensitive sample of all; beginning with the first hours of immersion, the size of the STPS sample was increased, showing the lowest dimensional stability.

TPS, MoTPS, and STPS water absorption rates reached a maximum water absorption of around 140%, while MaTPS maximum water absorption is 80%, as shown in fig.4.18; showing that cross-linking with malic acid improves starch water resistance significantly. This significant reduction is due to improved interfacial adhesion and an increase in polymer chain arrangements due to cross-linking [176]. In addition, MaTPS sample was dimensionally stable, even after 45 hours of wetting time, it didn't dissolve or form any cracks due to wetting, and there were no color stains observed on the sample. This result approves the effectiveness of cross-linking in improving the water sensitivity of starch [164]. The addition of succinic and malonic acid didn't significantly improve the water resistivity of starch.

The reduction in the absorbance curve seen in the graphs after about 21 hours of the wetting period is due to the dissolution of the sample in water. Glycerol and the starch molecules are absorbed to dissolve in the distilled water giving the remaining water a viscous nature.

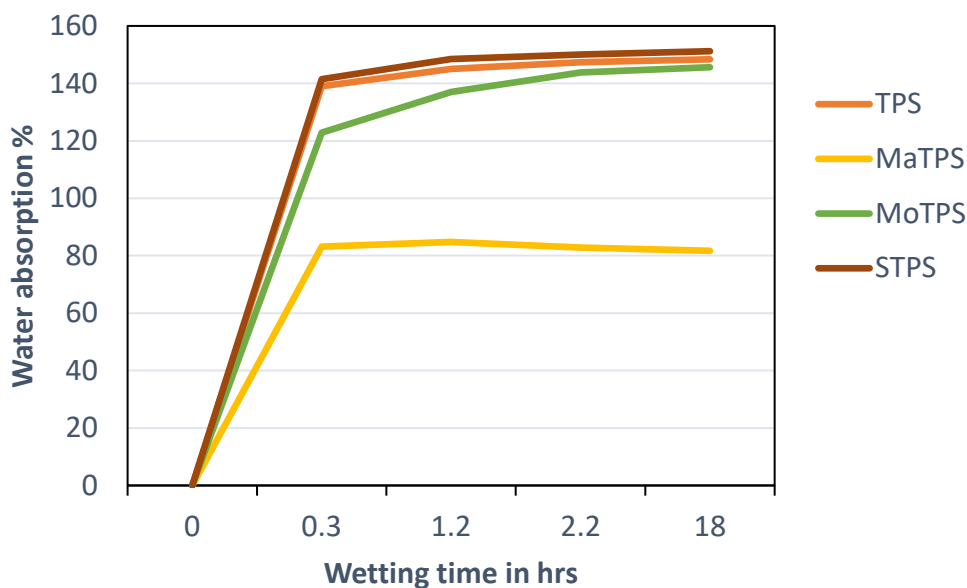


Figure 4. 18 Water absorption test results

#### 4.3.6 Summary

Starch film samples prepared using carboxylic acids, namely malonic acid, malic acid, and succinic acid as cross-linker, and glycerol and water as plasticizers were characterized and tested.

Test results revealed formation of ester as a result of cross-linking. Crosslinked samples were found to be less viscous. There is a high degree of cross-linking with malic acid as a cross-linking agent, followed by succinic and malonic acid. The maximum water absorption capacity of TPS, MoTPS, and STPS is found to be around 140%, and the water absorption capacity of MaTPS is 80%; this shows a considerable improvement in the water resistance of starch when cross-linked using malic acid.

Even though malonic acid is 16 percent more acidic and 36 percent more soluble than malic acid, the presence of one more hydroxyl functional group in malic acid has a greater influence on cross-linking. Increased carbon chain length on succinic acid has a negative effect on cross-linking reaction, which could be attributed to its effect on acidity and solubility reduction. An increase in the number of hydroxyl groups and acidity promoted cross-linking starch on the respective orders, whereas an increase in the carbon chain length has affected cross-linking negatively.



#### 4.4 Cross-linking of Starch Using Terephthalic acid and Pyromellitic acid dianhydride

Pyromellitic dianhydride (PMDA) is a key cross-linking agent utilized in the production of high-temperature-resistant polymers. Due to their reactivity with nucleophiles, such as the OH groups of starch structure, anhydrides are suitable cross-linkers. Because of the functionality group features, its commercial uses are expanding. Pyromellitic dianhydride is utilized as a monomer in the production of thermoplastics such polyesters, polyethers, plasticizers, and epoxy resins. One of the most significant applications of PMDA is the production of polyethylene terephthalate (PET) for food packaging materials [126].

TA and PMDA are utilized to modify the property of thermoplastic corn starch. These acids differ in their carboxyl functional group. TA has two carboxylic groups and PMDA has four carboxylic functional groups. Both terephthalic and Pyromellitic has a benzene ring in their molecular structure.

This experiment is intended investigate the effect of the molecular structure, the acidity condition during reaction, and ratio of PMDA and TA on corn starch crosslinking.

Table 4.6 shows the samples produced for this experiment and the type and ratio of the constituents.

Table 4. 6 Samples Produced and the Amount of the ingredients

S.No.	Designation	Corn Starch	Water	Glycerol	Terephthalic acid	Pyromellitic acid
1	TPS	8.0 g	200 ml	2.88 g	0	0
2	5PTPS	8.0 g	200 ml	2.88 g		0.4 g
3	5TeTPS	8.0 g	200 ml	2.88 g	0.4 g	
4	10PTPS	8.0 g	200 ml	2.88 g		0.8 g
5	10TeTPS	8.0 g	200 ml	2.88 g	0.8 g	
6	20PTPS	8.0 g	200 ml	2.88 g		1.6 g
7	20TeTPS	8.0 g	200 ml	2.88 g	1.6 g	
8	PTPS3	8.0 g	200 ml	2.88 g		1.6 g
9	PTPS5	8.0 g	200 ml	2.8 g		1.6 g
10	PTPS11	8.0 g	200 ml	2.88 g		1.6 g

#### 4.4.1 Fourier Transform Infrared Spectroscopy

The FTIR spectra of the PTMD crosslinked starch (PTPS) at different concentration and acidity and the terephthalic acid crosslinked starch (TeTPS) are shown in fig. 4.19. There are strong FTIR peaks at  $1728\text{ cm}^{-1}$ ,  $1725\text{ cm}^{-1}$ , and  $1738\text{ cm}^{-1}$  for the PTPS samples unlike for the TeTPS samples. These peaks reveal C=O stretching vibration caused by ester formation as a result of the cross-linking reaction. This shows that there is no or less crosslinking reaction between terephthalic acid and the starch. This could be due to the very limited solubility of terephthalic acids compared to Pyromellitic acid at  $90\text{ }^{\circ}\text{C}$ [177].

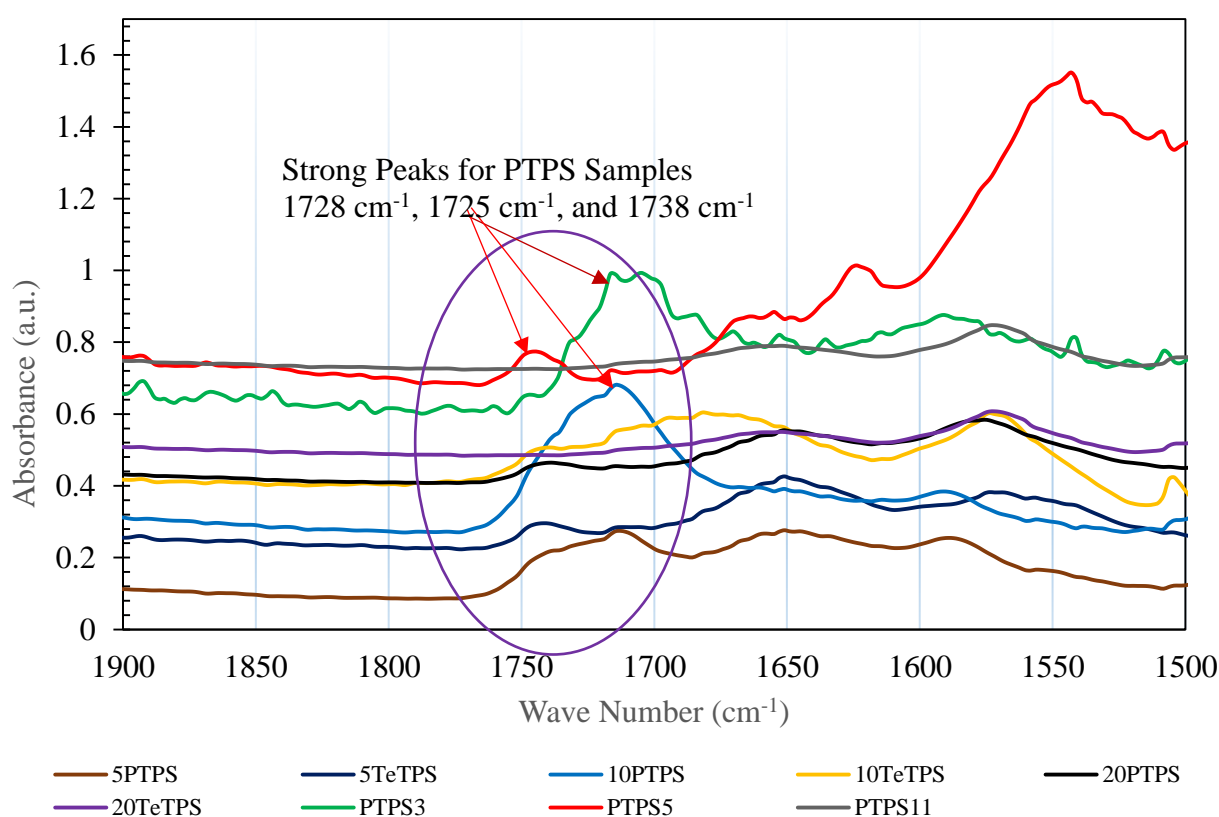


Figure 4. 19 Fourier Transform Infrared Spectroscopy (FTIR) Spectrum of samples

#### 4.4.2 X-Ray Diffraction

The angular diffraction pattern in fig.4.22 displays the terephthalic acid modified starch have strong diffraction peaks at  $17.5^{\circ}$  and  $28^{\circ}$  on other hand this diffraction peaks are not displayed on the PTPS samples. The PTPS samples exhibit less crystallinity as the result of the crosslinking reaction. The diffraction peaks pattern for the starch cross-linked with Pyromellitic dianhydride is almost flattened, indicating that the polymer is transitioning from crystalline to amorphous [34].

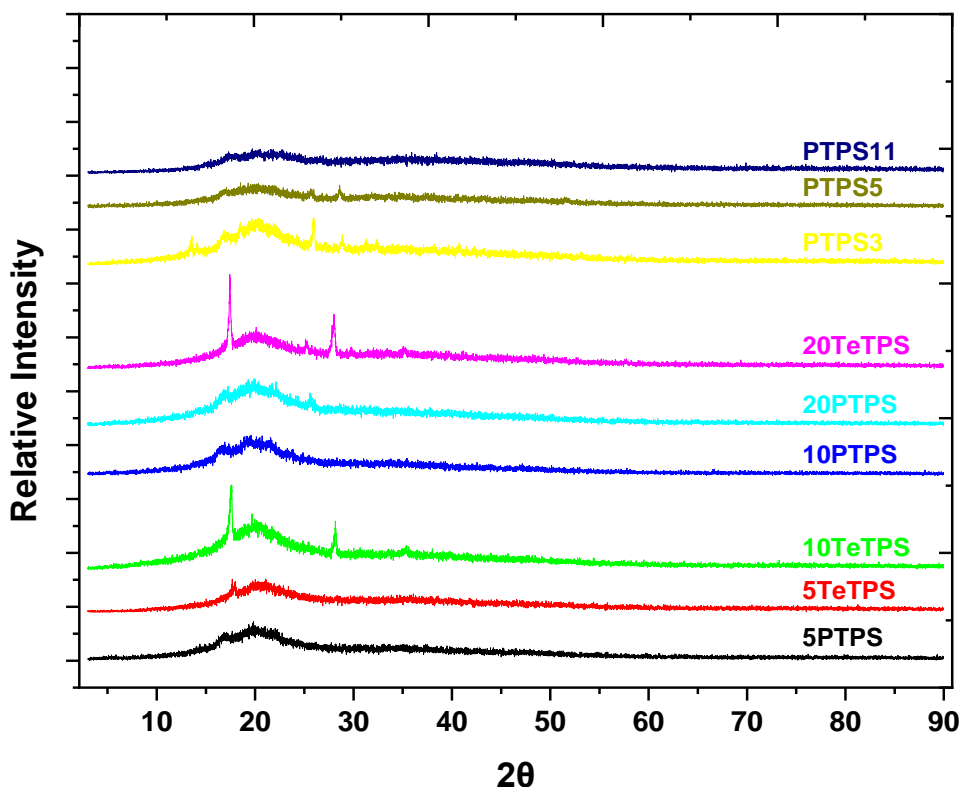


Figure 4. 20 X-Ray Diffractograms of modified starches

#### 4.4.3 Thermo-Gravimetric Analysis

Thermogravimetric plots of the modified starch samples are illustrated in fig. 4.23 below. In these figures, unlike the TPS and TeTPS, the thermograms obtained from Pyromellitic acid modified starch samples show similarities up to a temperature of 250 °C. TPS and TeTPS films exhibited four degradation steps, but the PTPS modified samples are degraded at three distinct steps. The first degradation step for TPS is 50-100 °C, which is the evaporation water. This step is also happening in TeTPS with a very slight difference. The second degradation step for PTPS (third for TPS and TeTPS) occurs from 280-340 °C representing the decomposition of starch. Beyond this temperature the Pyromellitic acid crosslinked starch samples show a very slow (inclined curve) decrease in weight which is a sign of stability. The terephthalic acid modified starch show almost similar (vertical curve) pattern with TPS till about 340 °C. Fig. 4.23 C show that PTPS11 a sample prepared at pH of 11, has less stability than PTPS 3 and PTPS 5. This indicates that the crosslinking reaction can produce stable crosslinked polymers at higher pH.

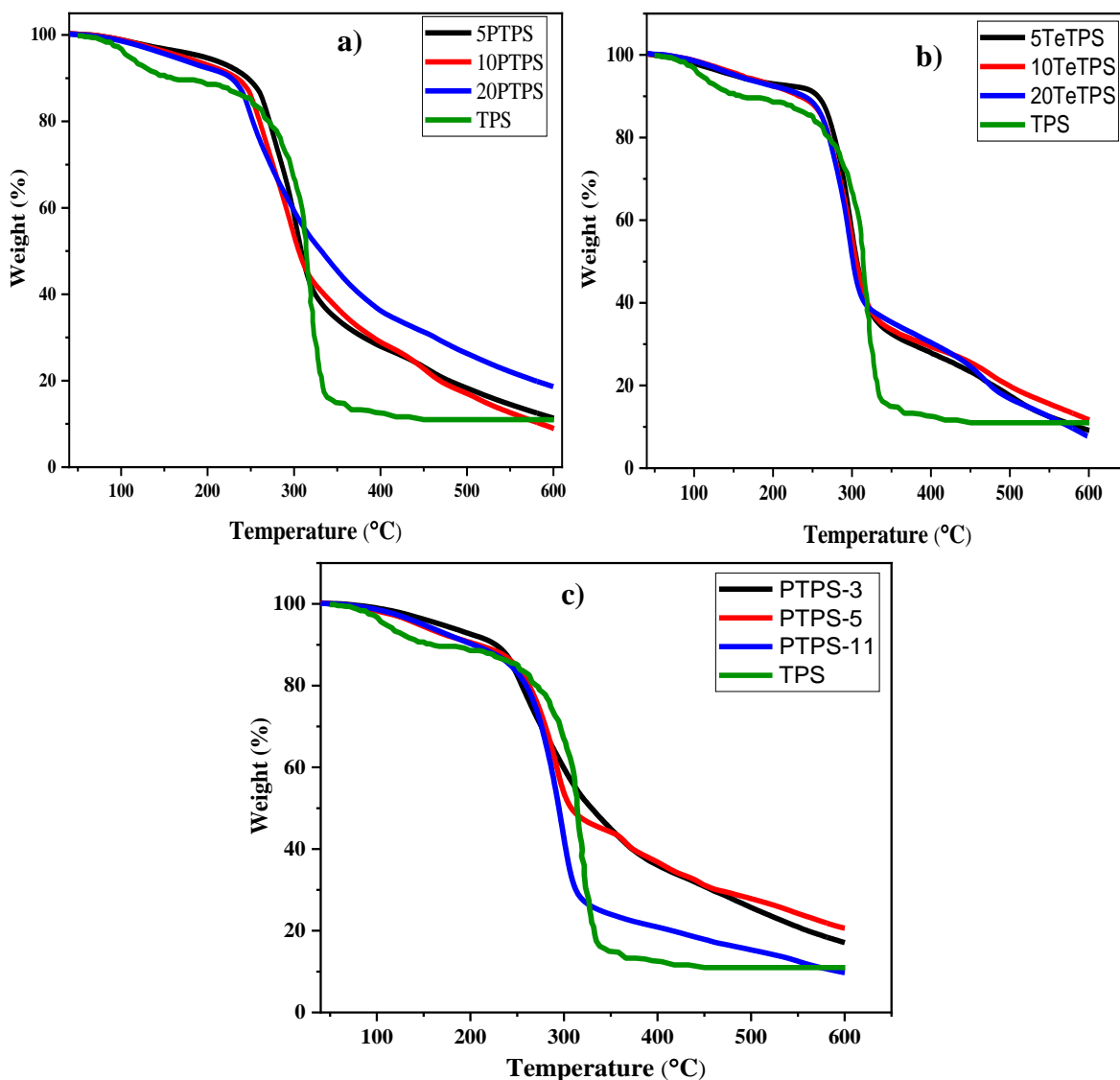
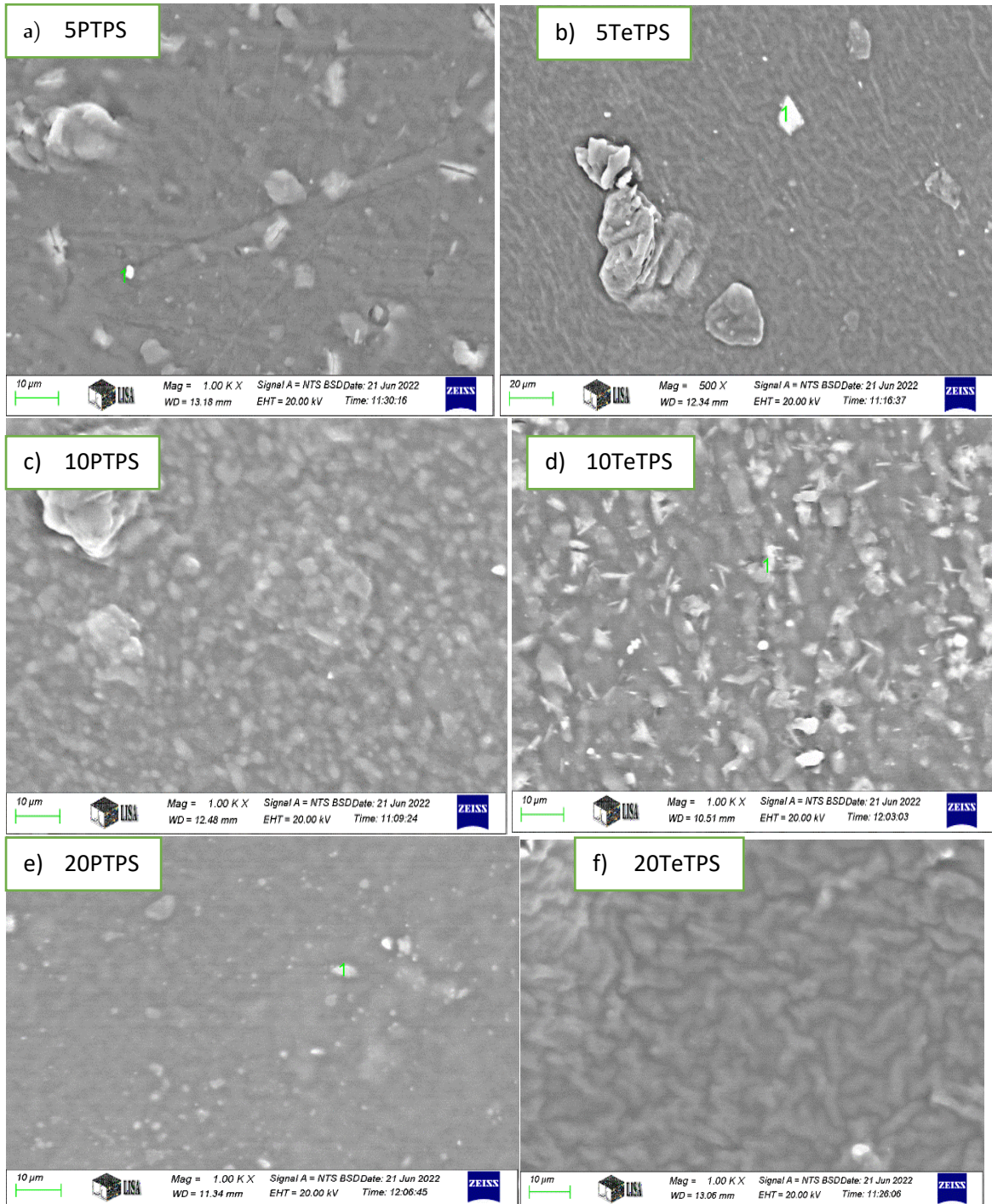


Figure 4. 21 TGA Thermographs of the Samples

#### 4.4.4 Surface Morphology

The surface morphology of the chemically modified corn starch samples shown in fig. 4.23 below illustrates that the Pyromellitic acid modified samples have relatively smooth surfaces compared to the terephthalic acid modified starch samples. It is also observed that the small amount of crosslinking agent the less is the coagulation and surface smoothness. The effect of the acidity of the reaction solution is that as the acidity increases, it means that there is less amount of NaOH added and contributes less to the impurity of the samples.





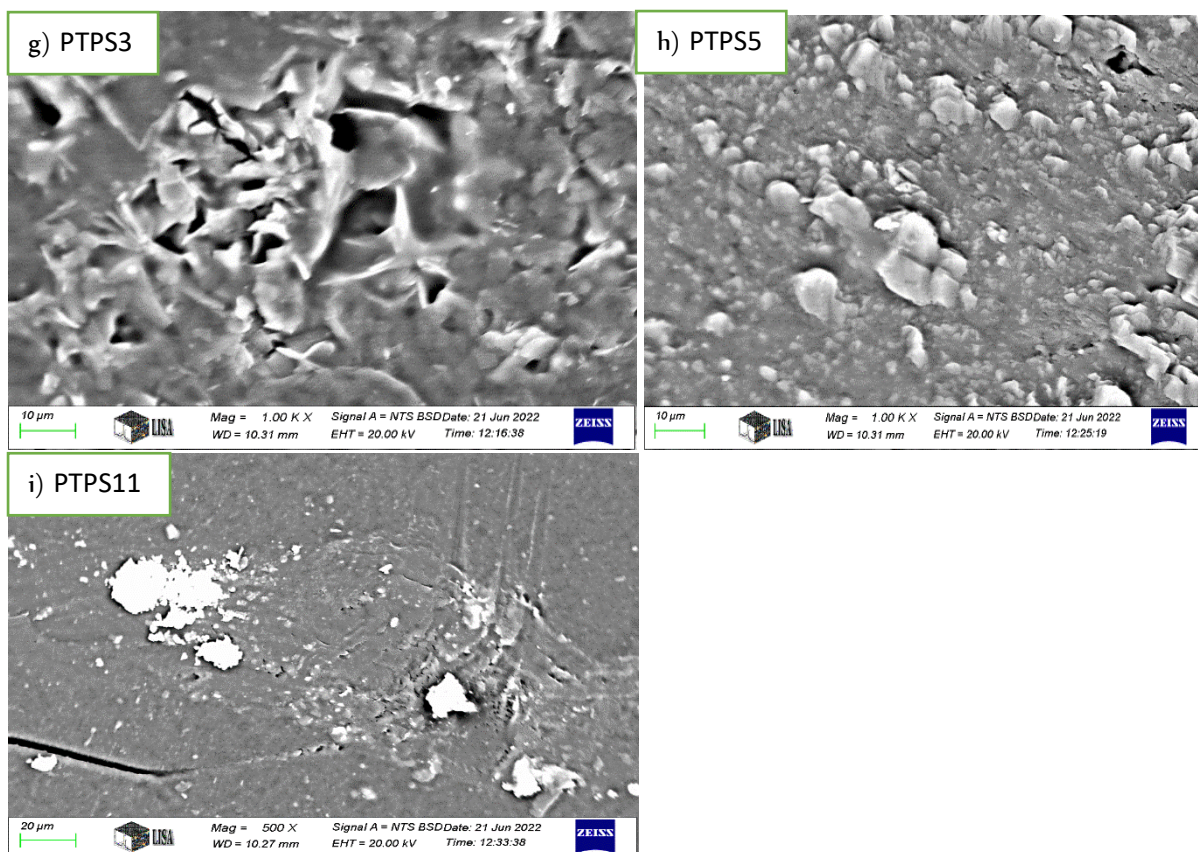


Figure 4. 22 Surface Morphology of Samples

#### 4.4.5 Stress-Strain Property

Table 4.7 shows the mechanical property of the samples. For teleptalic acid cross-linked starch there is a decrease in both tensile strength ( $2.0 \pm 0.1$  MPa,  $2.3 \pm 0.5$  MPa,  $2.0 \pm 0.3$  MPa) and maximum elongation at break ( $71.5 \pm 3.8\%$ ,  $87.7 \pm 9.5\%$ ,  $67.3 \pm 3.9\%$ ) compared to the TPS which is  $2.39 \pm 1.3$  MPa and  $108.6 \pm 7.7\%$  respectively. This is another result that revealed terephthalic acid has not crosslinked corn starch in addition to the FTIR result.

Compared to TPS; the pyromellitic acid crosslinked starch has shown better tensile strength of  $5.4 \pm 0.2$  MPa, and lower elongation at break of  $80.2 \pm 9.8\%$  due to additional bonding created during crosslinking that enhanced the strength and restricted the elongation.

It is also indicated that as the ratio of pyromellitic acid increases from 5% to 20% for 5PTPS, 10PTPS, and 20PTPS samples, the tensile strength decreases. This could be due to excess PMDA which could have converted to sodium salt or hydrolysed. The elongation at break is maximum for 10PTPS.

Table 4. 7 The Tensile Test Result of Samples

<b>Samples</b>	<b>Modulus (Automatic Young's)</b>	<b>Maximum Tensile Stress</b>	<b>Maximum Tensile Strain</b>
	(MPa)	(MPa)	(%)
TPS	10.8 ± 6.9	2.4 ± 1.3	108.6 ± 7.6
5PTPS	85.4 ± 6.1	5.4 ± 0.2	80.2 ± 9.8
5TeTPS	10.8 ± 1.8	2.0 ± 0.1	71.5 ± 3.8
10PTPS	28.6 ± 5.2	3.5 ± 0.4	97.8 ± 6.6
10TeTPS	14.3 ± 3.2	2.3 ± 0.5	87.7 ± 9.5
20PTPS	44.7 ± 1.3	3.1 ± 0.3	66.4 ± 4.5
20TeTPS	24.6 ± 2.9	1.9 ± 0.3	67.3 ± 3.9

#### 4.4.6 Water Absorption Property

The water absorption curves fig.23 shows very quick water uptake in the first twenty minutes of the first immersion. In almost every section of the absorption curve, starting from the immersion to the end of the experiment. Pyromellitic acid cross-linked starch exhibits less water absorption rate than terephthalic acid crosslinked starch (TeTPS). After 120 hours of wetting time, the water absorption rate of the TeTPS samples exceeds 150% whereas the water absorption rate PTPS samples is still below 150%. Beyond the 120 hours of wetting time, the TeTPS samples disintegrate while the PTPS samples display some sign of dissolution keeping their dimensional stability. It means that reacting starch with terephthalic acid has negatively affected the water resistance of starch. This could be due to the higher amount of carboxyl functional group of Pyromellitic acid compared to terephthalic acid. An increase in cross-links hinders water diffusion in the polymer network, restricts the movement of polymer chains, and compacts the structure [178].

Keeping the reaction conditions the same, PTPS3, PTPS5, 10PTPS (prepared at pH of 7.5), and PTPS11 are prepared at 3, 5, 7.5, and 11 pH respectively. The increase in water absorption rate for PTPS11 is due to the transformation of dianhydride to sodium salt that reduces the crosslinking reaction. PMDA the crosslinking reaction increases at the lower pH value, resulting in an increase in water resistivity in PTPS3 [127].

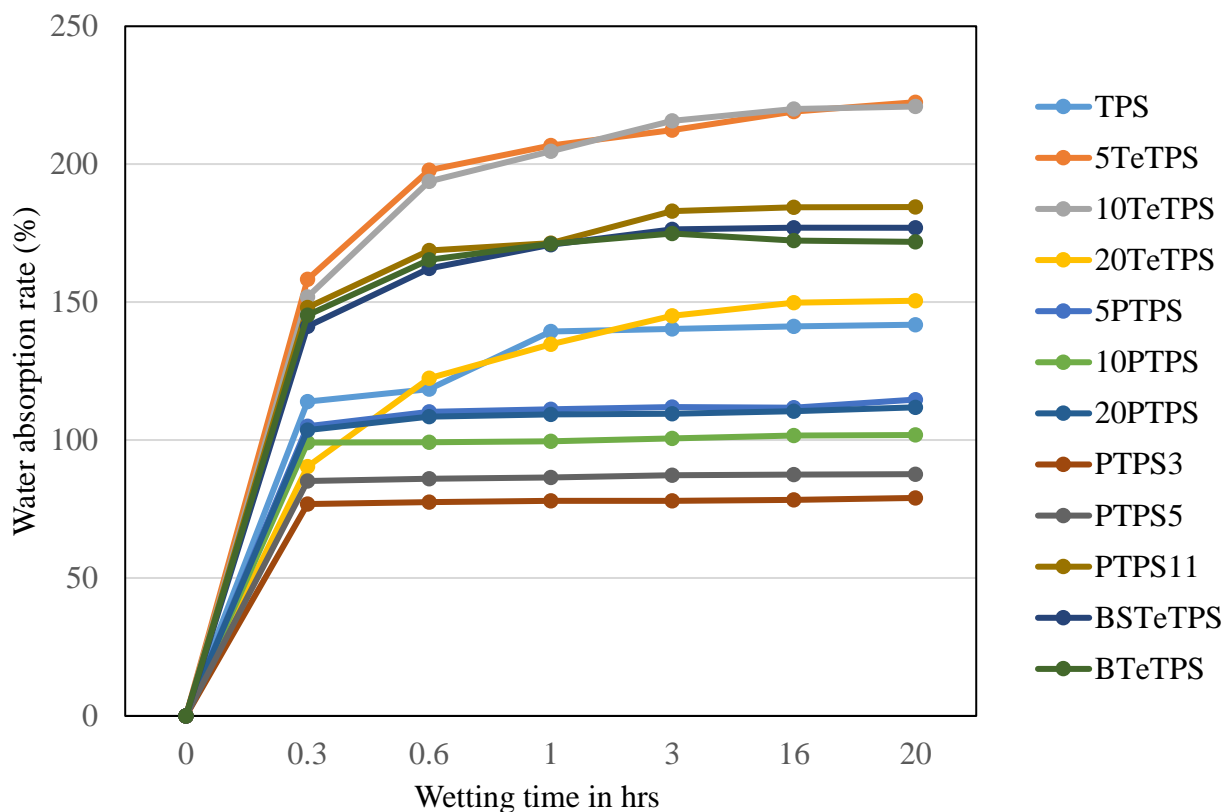


Figure 4. 23 Water Absorption Rate of Samples

#### 4.4.7 Summary

Corn starch was modified using terephthalic and Pyromellitic acids. The reaction was performed under 3, 5, 7, and 11 pH. The ratio of the modifying acids was varied from 5%, 10% and 20% (W/W) of corn starch. A thin film of modified crosslinked polymer was prepared; and tested and characterized for its mechanical, thermal, physical properties and change in molecular structure. The strong FTIR peaks at  $1728\text{ cm}^{-1}$ ,  $1725\text{ cm}^{-1}$ , and  $1738\text{ cm}^{-1}$  for the PTPS samples unlike for the TeTPS samples which indicates the ability of Pyromellitic acid and inability of terephthalic acid to crosslink corn starch.

The water absorption property of the samples indicates that after 120 hours of wetting time, the water absorption rate of the TeTPS samples exceeds 150% whereas the water absorption rate of PTPS samples is still below 150%. Beyond the 120 hours of wetting time, the TeTPS samples disintegrate while the PTPS samples display some sign of dissolution keeping their dimensional stability.

Unlike terephthalic acid cross-linked starch which shows no significant improvement due to lack of crosslinking; the tensile strength of property of pyromellitic acid dianhydride crosslinked



starch increased to  $5.43 \pm 0.18$  MPa, compared to tensile strength of TPS which is  $2.39 \pm 1.30$  MPa.

It is also indicated that as the ratio of pyromellitic acid increases from 5% to 20% for 5PTPS, 10PTPS, and 20PTPS samples, the tensile strength decreases.

Comparing the effect of the acidity of the reaction condition; PTPS5, a reaction done under 5 pH. Was found to have better property improvements.

From the thermogravimetric plots, three and four degradation phases are observed for TeTPS and TPS; and PTPS respectively; indicating a sign of thermal stability to the PTPS samples 10 PTPS, corn starch modified using 10% (W/W) ratio of Pyromellitic acid was found an optimal concentration.

#### 4.5 Thermoplastic Corn Starch Reinforced with Pinewood Fibre and Calcium Carbonate Precipitate Filler

In this study; plasticizing the corn starch, mixing with calcium carbonate and pinewood fibre, heating and pressing were the successive steps followed to produce the required samples. The samples were characterized for their mechanical and physical properties. Table 4.8 indicates the samples prepared for this specific study.

Table 4. 8 Samples Prepared and Raw Materials Used for Sample Preparation

S.No.	Sample Name	Description	Composition in Weight Percent
1	FTPS	Thermoplastic Corn Starch	60% corn starch + 40% glycerol
2	WTPS	Thermoplastic Corn Starch + Wood Fiber	60 % corn starch + 5% wood fiber +40% glycerol
3	CaTPS	Thermoplastic Corn Starch +Calcium Carbonate Precipitate (Micro, Not Fully Nano)	60% corn starch + 5% Calcium Carbonate Precipitate + 40% glycerol
4	HTPS	Thermoplastic Corn Starch + Wood Fiber +Calcium Carbonate Precipitate	60% corn starch + 5% Calcium Carbonate Precipitate + 5% wood fiber + 40% glycerol

### 4.5.1 Thermal Analysis

The DSC test result of all the samples is depicted in Fig.4.24 as a heat flow versus temperature thermogram. The DSC graph of free TPS and the composite samples indicates that there is a big variation in temperature at which the free TPS and other composite transform to another phase. The melting point of the composites is shifted to a lower temperature for the hybrid composite compared with the other composites. It means the addition of filler and fiber together has brought a major change on the melting point of TPS. Fig.4.24 also shows that the thermogram for TPS is smoother than the other composites but major changes on melting point are not observed when compared with CaTPS and WTPS.

The curves in the thermogram Fig.4.25 displays that the glass transition temperature ( $T_g$ ) has increased for both HTPS and WTPS, this might be due to the compatibility of wood fiber to create better interfacial adhesion with TPS. Addition of filler calcium carbonate precipitate doesn't have a significant effect on the glass transition temperature of the TPS composite. The thermogram below also depicts that the storage modulus decrease with increase in the temperature. The decrease in storage modulus is sharper in free TPS and CaTPS than HTPS and WTPS.

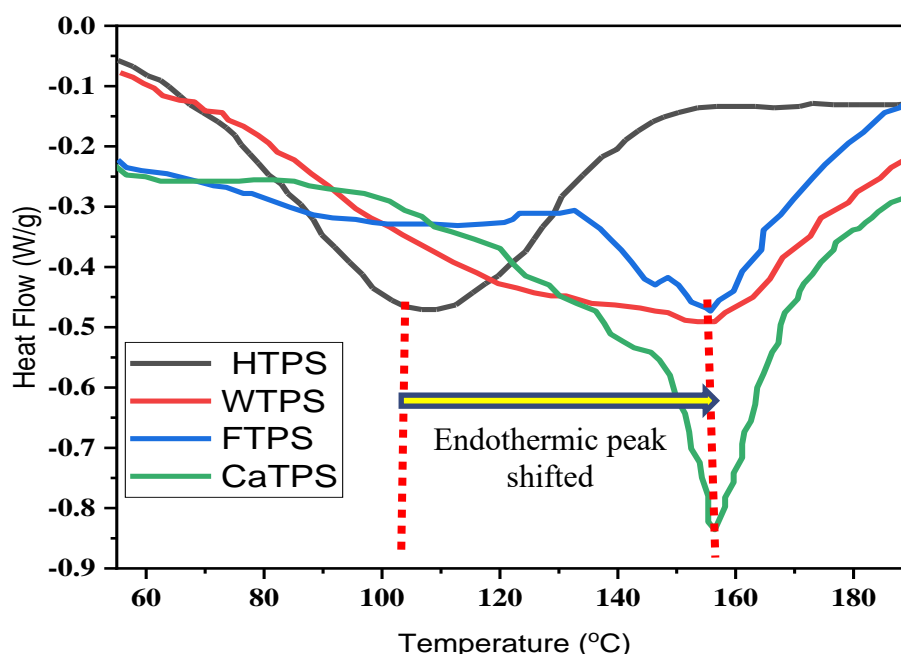


Figure 4. 24 DSC Thermogram of Composite Samples

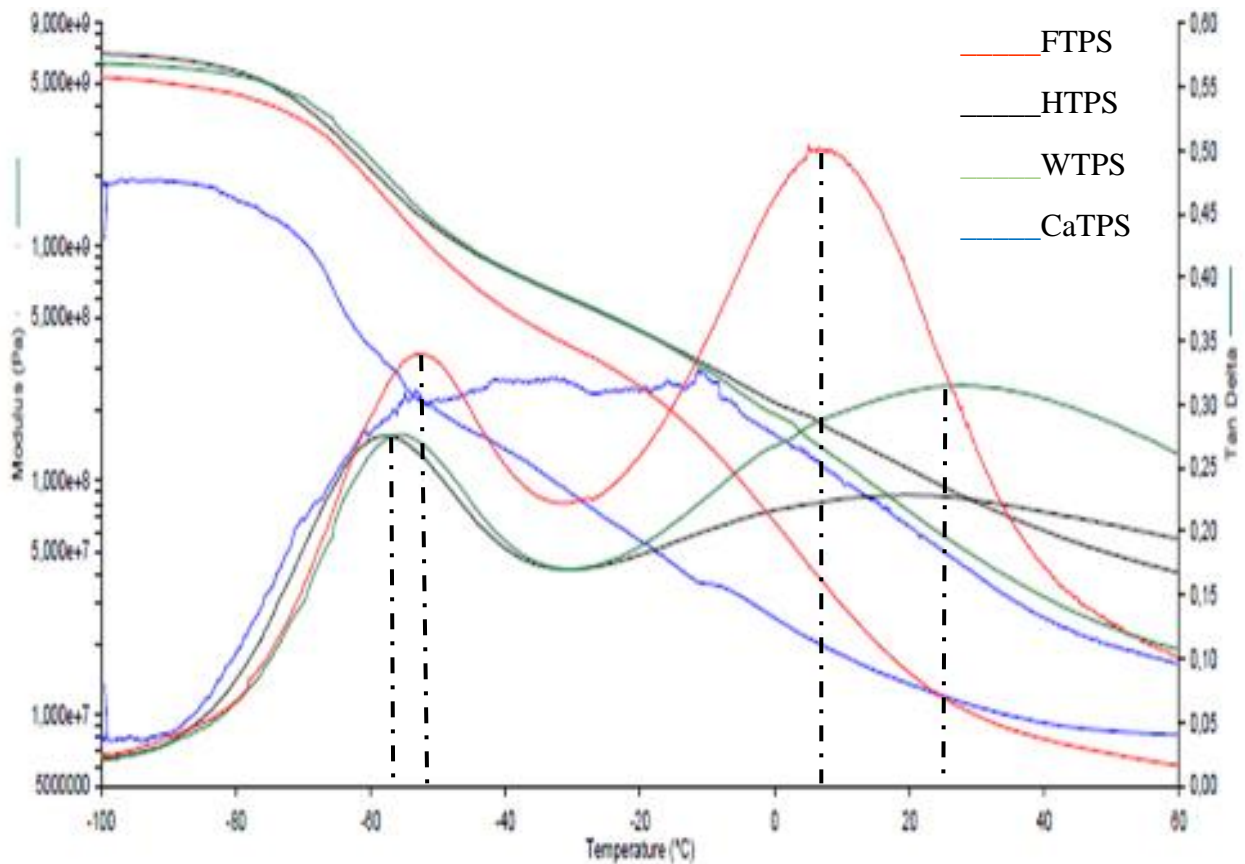


Figure 4. 25 DMA Result of Composite Samples

#### 4.5.2 Tensile Property of the Composites

Fig. 4.26 below shows the tensile stress test result of the four composites. WTPS reveals greater strength of 1.02 MPa which is a huge improvement compared to TPS, HTPS and CaTPS. This is due to the homogeneity and compatibility (both are hydrophilic). Calcium carbonate precipitate filled composite is found less strong due to the hydrophobicity of the filler compared to corn starch that leads to agglomeration.

The tensile strain graph shows that free TPS possesses a tensile strain of 70.87% which is better compared to than the other composite samples and decreases with the addition of wood fibre and/or filler. This is due to the fact that intercalation of fillers into starch promotes the formation of a restricted environment against the movement of polymer chains [12].

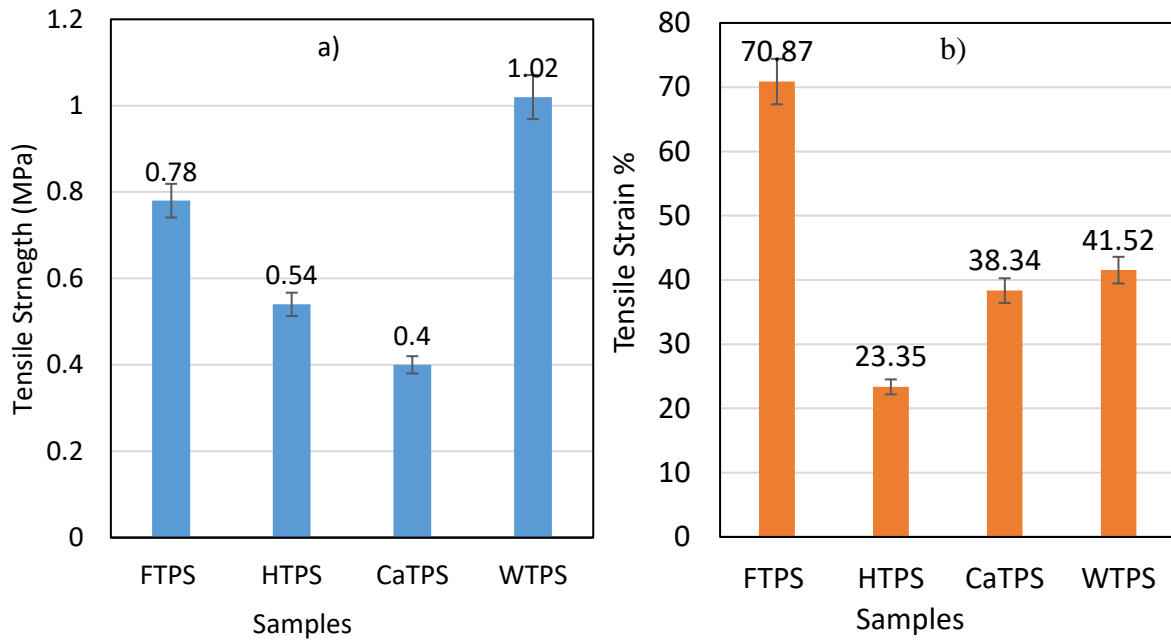


Figure 4. 26 a) Tensile Strength b) Tensile Strain Test Result of Composite Samples

#### 4.5.3 Hardness Property of the Composite Samples

The graph in fig.4.27 below shows that the hybrid composite HTPS has greater shore hardness of 41 kg/mm<sup>2</sup> followed by wood reinforced TPS. These facts could be attributed to the better interfacial adhesion of the TPS matrix with pine wood fibre reinforcement. The decrease in the hardness of the composite with calcium carbonate precipitate might be due to agglomeration of the filler that leads to inhomogeneity.

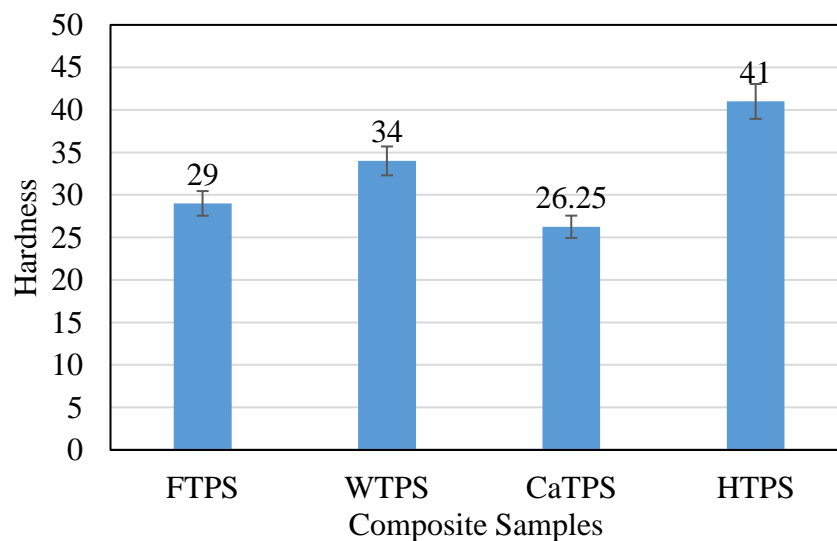


Figure 4. 27 Shore Hardness Test Result of the Composites

#### 4.5.4 Morphology of the Composite Samples

The SEM result shown in fig. 4.28 indicates agglomerations in the composites filled with the calcium carbonate precipitate (CaTPS) and the hybrid one (HTPS), but the wood fibre reinforced composite (WTPS) has got better homogeneity. This can be attributed to the hydrophobicity of the calcium carbonate precipitate which hinders the uniform distribution in the composite.

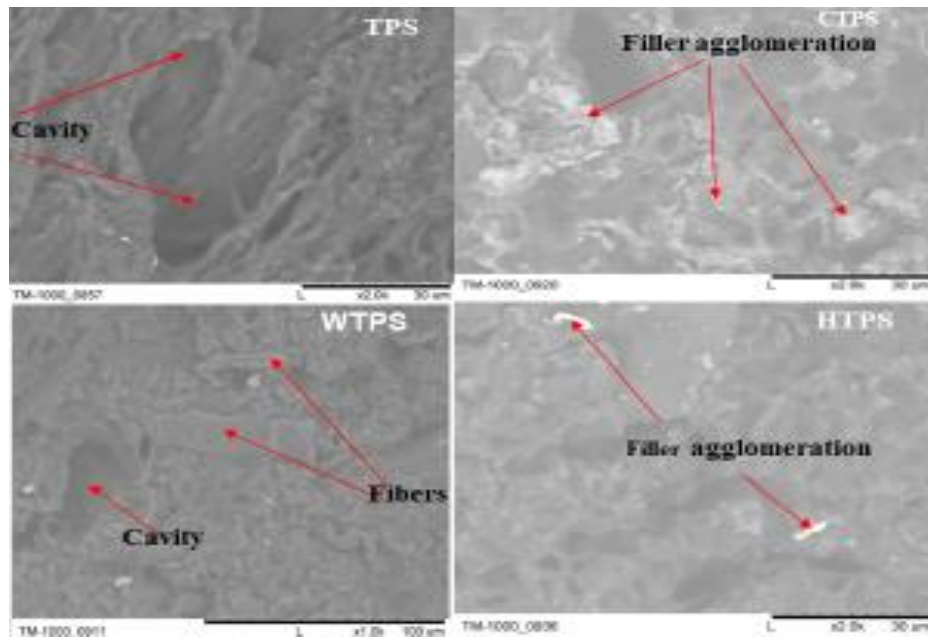


Figure 4. 28 SEM Test Result of the Composites

#### 4.5.5 Water Absorption Properties

As indicated in fig.4.29 below, FTSP was found water-sensitive and exhibit higher water absorption rate, followed by CaTPS due to agglomeration of the filler. HTPS exhibits a maximum water absorption rate of less 90% followed by WTPS. This indicates that reinforcing TPS with cellulosic fibre and calcium carbonate filler improves the water absorption. The wood fibre could have helped in in the homogenous distribution of the filler. Which is not exhibited with the filler alone. All the composite samples started dissolving in the distilled water after 3 hours of the testing period.

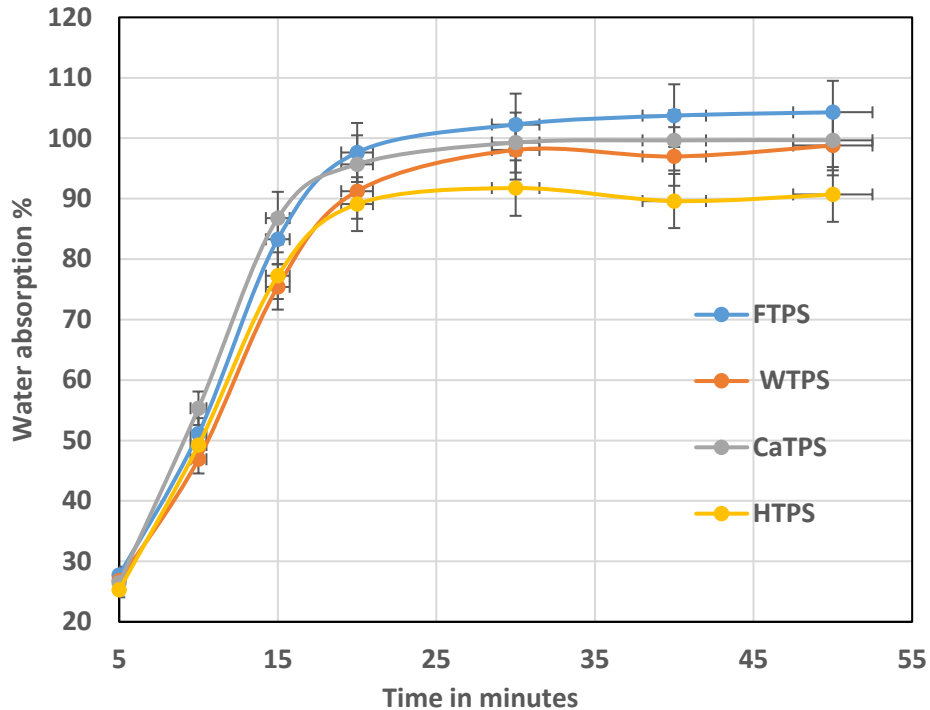


Figure 4. 29 Water Absorption Test Result of the Composites

#### 4.5.6 Summary

TPS composites reinforced by pine wood fibre and calcium carbonate precipitate were prepared by pressure moulding. WTTPS reveals greater strength of 1.02 MPa which is a huge improvement compared to TPS, HTPS and CaTPS. Free TPS possesses a tensile strain of 70.87% which is better compared to the other composite samples and decreases with the addition of wood fibre and/or filler. The hybrid composite HTPS has greater hardness of 41 kg/mm<sup>2</sup> followed by wood reinforced TPS. These facts could be attributed to the better interfacial adhesion of the TPS matrix with pine wood fibre reinforcement. The decrease in the hardness of the composite with calcium carbonate precipitate might be due to agglomeration of the filler that leads to inhomogeneity.

HTPS exhibits a maximum water absorption rate of less 90% followed by WTTPS which is comparably better than the TPS. This indicates that reinforcing TPS with cellulosic fibre and calcium carbonate filler improves the water absorption resistance of thermoplastic starch.

Results showed that pine wood fibre reinforced TPS sample has better water resistance, mechanical and morphological properties compared to pro precipitated calcium carbonate reinforced composites and the hybrid composites, this is due to the compatibility nature of wood fibre with starch.

## 4.6 Tartaric Acid Crosslinked Hybrid Starch Composites

With an aim to improve the mechanical, thermal and the water resistance property of starch; starch composite samples were prepared from Starch as matrix, sodium montmorillonite clay (Mc) and termite clay (Tc) as fillers and Enset fiber as reinforcement. Samples with composition described in table 4.9 are prepared, characterized, tested and interpreted.

Table 4. 9 Composite Starch Film Samples Prepared

S.No.	Designation	Corn Starch (g)	Water (ml)	Glycerol (g)	Tartaric acid (g)	Montmorillonite Clay (g)	Termite clay (g)	Fiber (g)
1	TPS	8.0	200	2.88	0	0	0	0
2	TaS	8.0	200	2.88	1.6	0	0	0
3	TaMcS	8.0	200	2.88	1.6	0.4	0	0
4	TaFS	8.0	200	2.88	1.6	0	0	0.16
5	TaTcS	8.0	200	2.88	1.6	0	0.4	0
	TaFMcS	8.0	200	2.88	1.6	0.4	0	0.16
7	TaFTcS	8.0 g	200	2.88	1.6	0	0.4	0.16

### 4.6.1 Fourier Transform Infrared Spectroscopy

Fig. 4. 30 Shows the FTIR spectra of the samples crosslinked with tartaric acid, filled with clay and reinforced with cellulosic fiber (Enset fiber).

The formation of new peak at  $1742\text{ cm}^{-1}$  on the FTIR spectrum for the samples prepared under the addition of tartaric acid indicates that reaction has occurred between tartaric acid and starch. The peak  $1742\text{ cm}^{-1}$  reveals C=O stretching vibration due to the formation of ester as a result of crosslinking. All the samples except TPS have shown this peak which tells that the crosslinking is mainly due to the reaction between starch and tartaric acid.

The band  $2929\text{ cm}^{-1}$  is due to the symmetric and asymmetric vibration of C-H bond. The broad band  $3298\text{ cm}^{-1}$  is assigned to the hydroxyl groups of starch.

Peaks in the range of  $993\text{-}1263\text{ cm}^{-1}$  are expected to occur due to stretching vibration of C-O bond. The peak at  $1604\text{ cm}^{-1}$  might be assigned to the water adsorbed by the starch molecules. Consequently, thermoplastic starch (TPS) and the tartaric acid, filler and cellulosic

reinforcement modified starch have shown difference in their chemical composition due to the reaction that happened in starch, tartaric acid and cellulosic fiber.

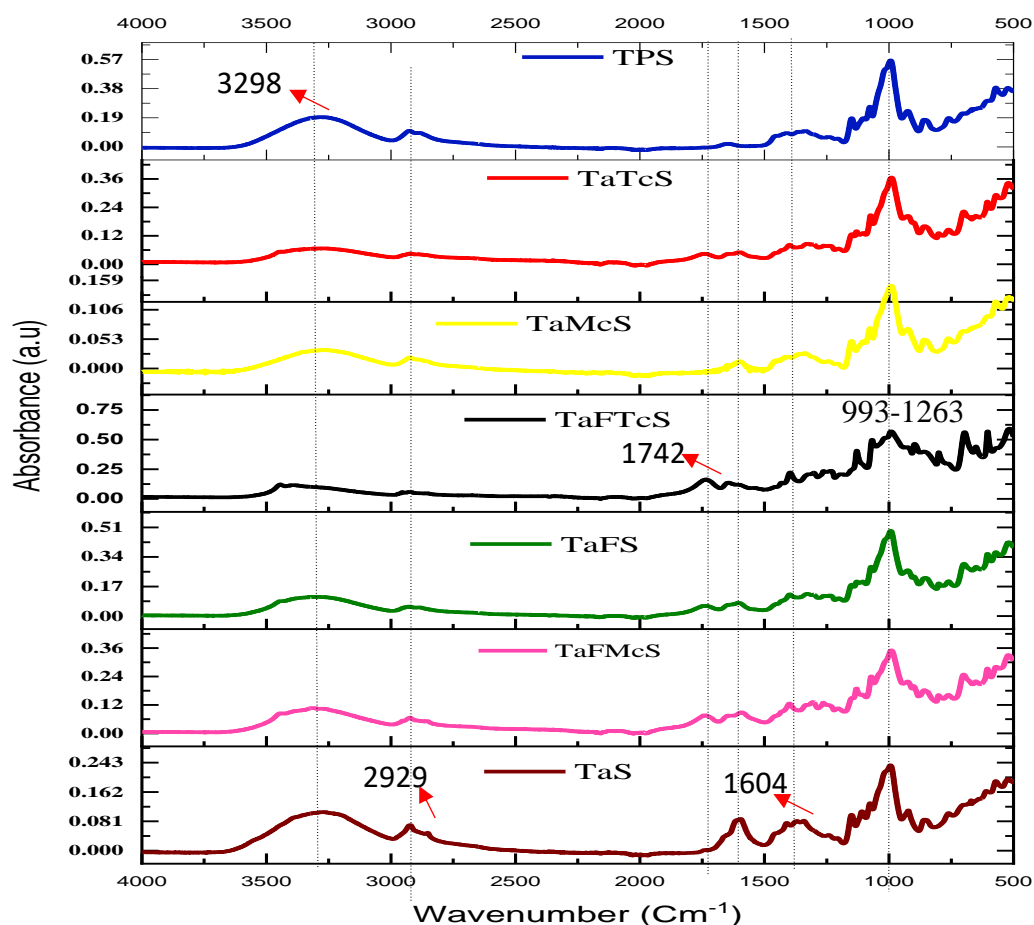


Figure 4. 30 Fourier Transform Infrared Spectroscopy (FTIR) Spectrum of samples

#### 4.6.2 X-Ray Diffraction

Corn starch is a naturally semi-crystalline material made up of amylose and amylopectin having sharp diffraction peaks in the range of 15-24° (2θ).

As is seen in the diffractograms fig. 4.31 Thermoplastic starch (TPS) and tartaric acid crosslinked thermoplastic starch (TaS) both lacks diffraction peaks. This is because, in the crosslinking and gelatinization process the hydrogen bonds of starch are destructed by glycerol, water and tartaric acid entering in to the crystalline starch granules.

Samples modified using clay, TaMcS and TaTcS do not show strong diffraction peaks. This might be due to the intercalation and exfoliation of glycerol and tartaric acid molecule inside the clay layers.



The starch film samples fabricated using cellulosic fibers as reinforcement; TaFS, TaFMcS and TaFTcS shows sharp diffraction peaks at  $21.3^\circ$ ,  $31^\circ$  which might be due to the reaction of cellulosic molecules and starch molecules which later crystallizes after the solution is precipitated.

Thus, addition of micro clays as fillers and crosslinking with tartaric acid has produced starch film samples with high amorphous structure than those reinforced with cellulosic natural fibers.

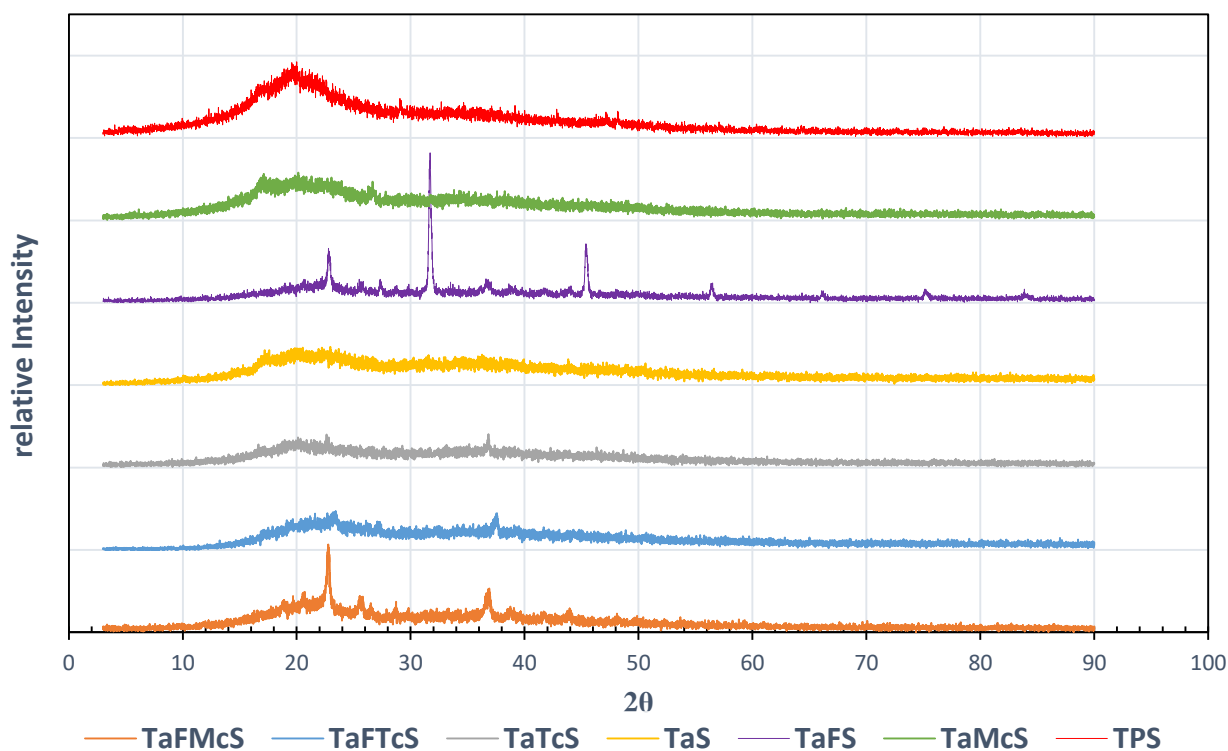


Figure 4. 31 X-Ray Diffractograms of Starch Composites

### 4.6.3 Surface Morphology

The morphology of the starch film samples prepared adding tartaric acid as a cross-linker, montmorillonite and termite clay as fillers and cellulosic fiber as reinforcements and control sample TPS are studied by Scanning electron microscope (SEM) and the results are presented in fig. 4.32. Comparing the surface morphology of control TPS and TaS (tartaric acid crosslinked TPS), TaS shows more uniformity and homogeneity than TPS as a result of crosslinking. The addition of fiber as reinforcement in TaFTcS, TaFS, and TaFMcS seems to affect the samples create wider pores, fluffy structure which has largely affected the water uptake of the samples. The cave like structure in the TaMcS sample is the result of coagulation

of montmorillonite clay. Generally, Crosslinking has modified the size and aggregation of the starch molecules.

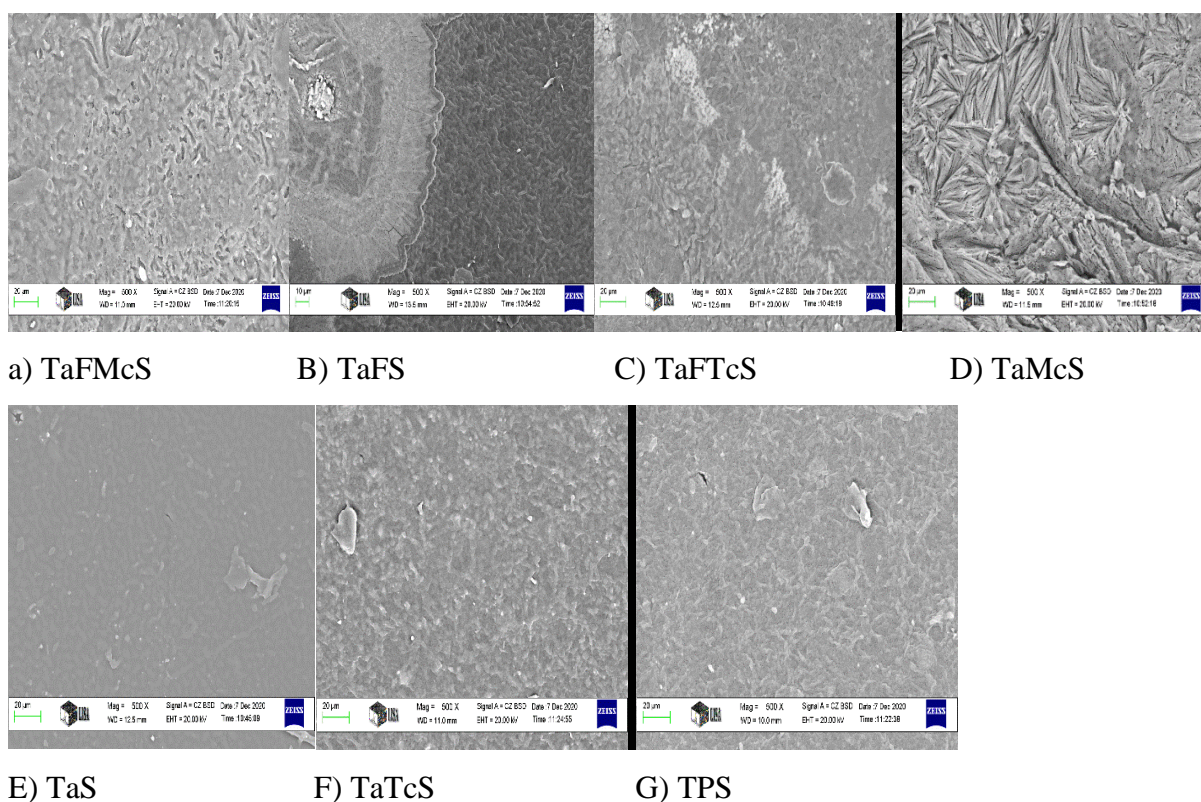


Figure 4.32 SEM Scans of Samples

#### 4.6.4 Dynamic Mechanical Analysis

Fig. 4.33 below shows the dynamic mechanical analysis result of the prepared samples. Storage modulus shows the elastic behavior of materials under range of temperature. As is clearly seen in fig. 4.32 below the storage modulus decreases as temperature increases.

The  $\tan \delta$  curve shown in the fig. 4.32. Generally is lower for TPS than all of the samples. The increase on the  $\tan \delta$  value on the modified samples is the result of mainly crosslinking of starch by tartaric acid. It means that the modified samples has got increased viscosity than TPS. The storage modulus ( $E'$ ) of the samples has decreased as the temperature increases from -40 to 60, but there is no clear variation on the stiffness of the samples which will require further experimentation.

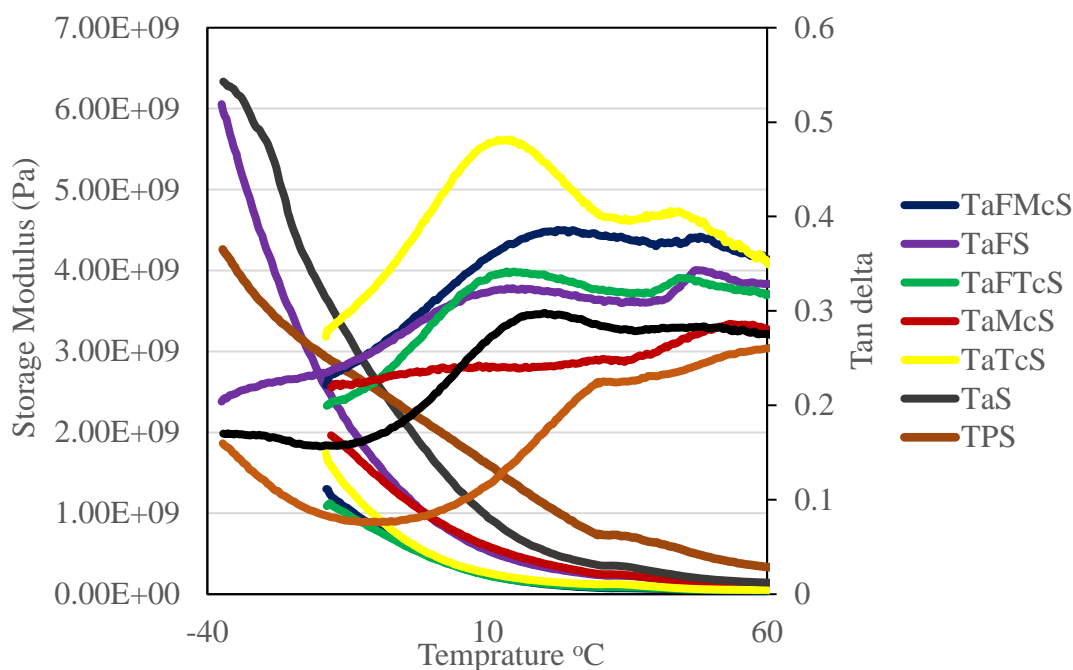


Figure 4. 33 Dynamic Mechanical Analyzer (DMA) Thermogram of Samples

#### 4.6.5 Mechanical Property (Stress-Strain Test)

Table 4.10 below shows the tensile stress-strain property of the modified corn starch samples. The maximum tensile stress and elongation at break of tartaric acid crosslinked starch samples are 4.38 MPa and 73.51% respectively. The maximum tensile stress and maximum tensile strain (elongation at break) of TPS is 2.29 MPa and 118% respectively, and is found to have maximum tensile strain and relatively minimum tensile stress than the other samples. The elongation at break of tartaric acid crosslinked starch (TaS) has reduced by half compared to TPS while the tensile strength is almost doubled. Tartaric acid cross-linking, inclusion of clay fillers and cellulosic fiber has affected the increase in stacking of molecular structure, densification of interstitial spaces and cohesion of starch molecules respectively. This has caused the increase in tensile strength and the reduction in elongation at break.

Table 4. 10 Tensile Test Result of Samples

Samples	Maximum Tensile stress	Maximum Tensile strain
	(MPa)	(%)
TaMcS	$3.9 \pm 0.5$	$50.5 \pm 5.2$
TaFS	$2.6 \pm 0.3$	$45.6 \pm 3.9$
TaFMcS	$2.3 \pm 0.2$	$36.6 \pm 4.2$
TaS	$3.4 \pm 0.3$	$73.5 \pm 5.6$
TaFTcS	$3.2 \pm 0.4$	$37.1 \pm 5.8$
TaTcS	$3.1 \pm 0.2$	$64.5 \pm 9.3$
TPS	$2.3 \pm 0.5$	$117.8 \pm 10.2$

#### 4.6.6 Water Absorption Property

Fig. 4.34 shows that all the samples have high absorption rate in the first 20 minutes of wetting time. TaTcS, tartaric acid crosslinked starch filled with termite (iron rich kaolinite) clay filler, is found to be the composite with highest water absorption rate among all the samples fabricated. This might be due to the coagulation effect of the clay opening cracks on the film so that water can easily penetrate. TPS, non-crosslinked thermoplastic starch, is also found to be water sensitive next to TaTcS sample. This result approves the effectiveness of crosslinking on improving the water sensitivity of starch. Fig.4.34 also shows that TaS (tartaric acid crosslinked thermoplastic starch) and TaMcS (tartaric acid crosslinked TPS filled with sodium montmorillonite clay) are the samples with improved water sensitivity; having maximum water absorption rate of about 85% and 100% respectively which a huge difference compared to TPS and TaTcS which is 140% and 160% respectively. This is due to the crosslinking effect of tartaric acid and the uniform distribution of the filler on starch film which hinders the passage of water. Reinforcing of thermoplastic starch with treated cellulosic fibers did bring significant change on water sensitivity of the samples as can be shown for the samples TaFS, TaMcFS and TaTcFS compared to TPS. Generally, crosslinking of TPS with tartaric acid has significantly improved the water sensitivity of starch. Adding montmorillonite clay as a filler also improved the water sensitivity to some extent.

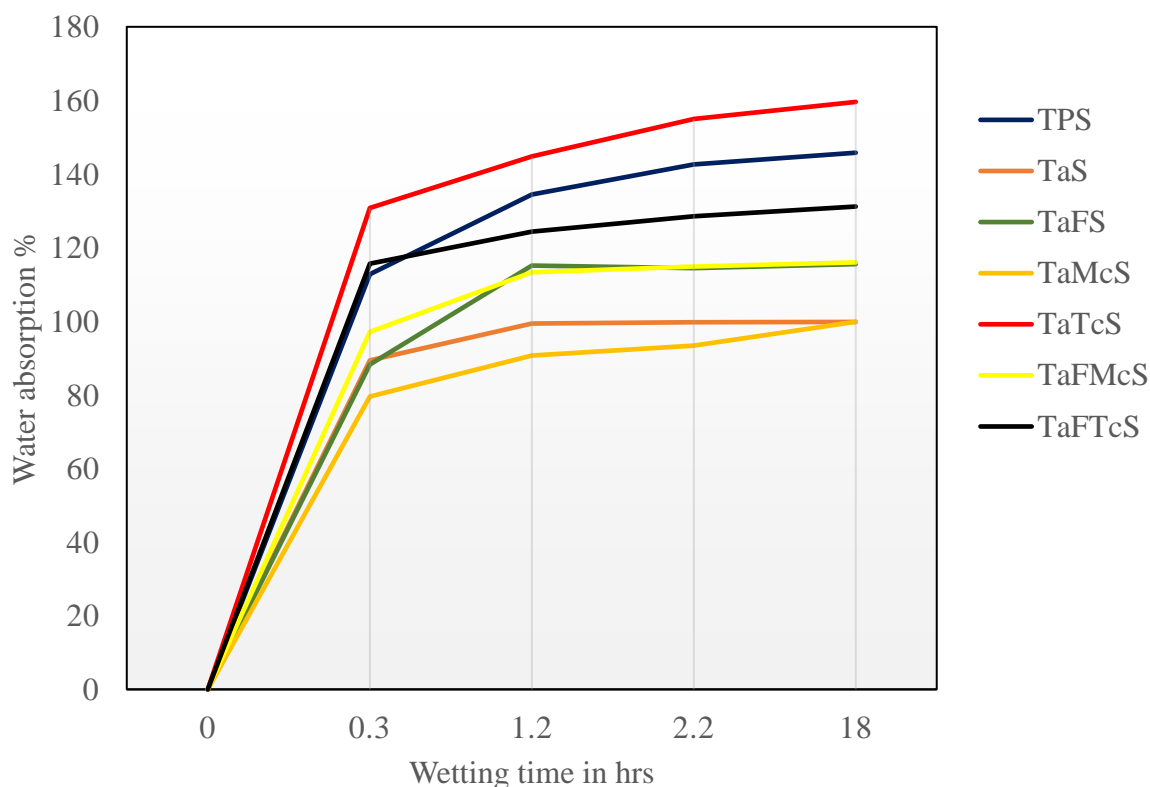


Figure 4. 34 Water Absorption Test Result

#### 4.6.7 Summary

Starch film samples prepared using tartaric acid as cross-linker, sodium montmorillonite clay, termite clay (iron rich kaolinite) as fillers, cellulosic natural fiber (inset fiber) as reinforcement; and glycerol and water as plasticizers were characterized and tested.

The formation of new peak at  $1742\text{ cm}^{-1}$  on the FTIR spectrum for the samples prepared under the addition of tartaric acid indicates that crosslinking reaction has occurred between tartaric acid and starch.

The maximum tensile stress and elongation at break of tartaric acid crosslinked starch and TPS samples were found to be 4.38 MPa and 73.51%; and 2.29 MPa and 118% respectively. The elongation at break of tartaric acid crosslinked starch (TaS) has reduced by half compared to TPS while the tensile strength is almost doubled.

TaS (tartaric acid crosslinked thermoplastic starch) and TaMcS (tartaric acid crosslinked TPS filled with sodium montmorillonite clay) have maximum water absorption rate of about 85% and 100% respectively which a huge improvement compared to TPS and TaTcS which is 140% and 160% respectively.

Thus, the tartaric acid crosslinked starch (TaTPS) has got better water resistance, and tensile strength than TPS, which is considered good property for packaging materials. Addition of clay fillers didn't significantly improve the water resistivity due to coagulation. Reinforcing of starch with cellulosic natural fibers has also negatively influenced the water resistivity even if it has significantly improved the strength.

The hybrid composites of starch, clay, and insert fiber has also improved the mechanical property of starch, but reduced the water resistance. Thus, among the sample composites characterized and tested, TaS was found to have optimal behavior for packaging applications.

## **The Most Important Observations of the Work**

### **Thesis 1**

We have effectively produced cross-linked corn starch polymer thin film using carboxylic acids as a cross-linker optimizing the process parameters. We investigated that availability of plasticizer is a critical factor for the cross-linking reaction and the plasticity property of the thin polymer film. We examined that the addition of sodium hydroxide before the addition of acids helped the swelling of the starch. The swelling happens due to breakdown of the intermolecular hydrogen bonds and the formation of new hydrogen bonds with water to form a larger, open structure. Alkali helps the de-bonding process by chain scissoring and helps the formation of new bonds within adjacent chains at the same time. This type of bond formation could help to improve the tensile properties of the materials

The appearance of new absorbance peaks on the FTIR spectrum for ester functional group; the decrease in number and intensity of the diffraction peaks on the X-Ray diffractograms and degree of crystallinity, including water absorption have proved that crosslinking has occurred.

## Thesis 2

Number of OH group per molecule, Acidity and carbon chain length ( $\text{CH}_2$ ) of carboxylic acids were investigated for their effects on crosslinking potential of thermoplastic starch. We investigated that the presence of an additional OH group in the carboxylic acids has helped form more hydrogen bonding between starch molecules. Acidity of the carboxylic acids is found to be a factor for better cross-linking potential. The increase in carbon chain length ( $\text{CH}_2$ ); has negatively affected its cross-linking potential. Thus, among the three variables studied, we investigated that increase in the number of hydroxyl groups and acidity promoted cross-linking of starch on the respective orders, whereas increase in the carbon chain length has affected cross-linking negatively.

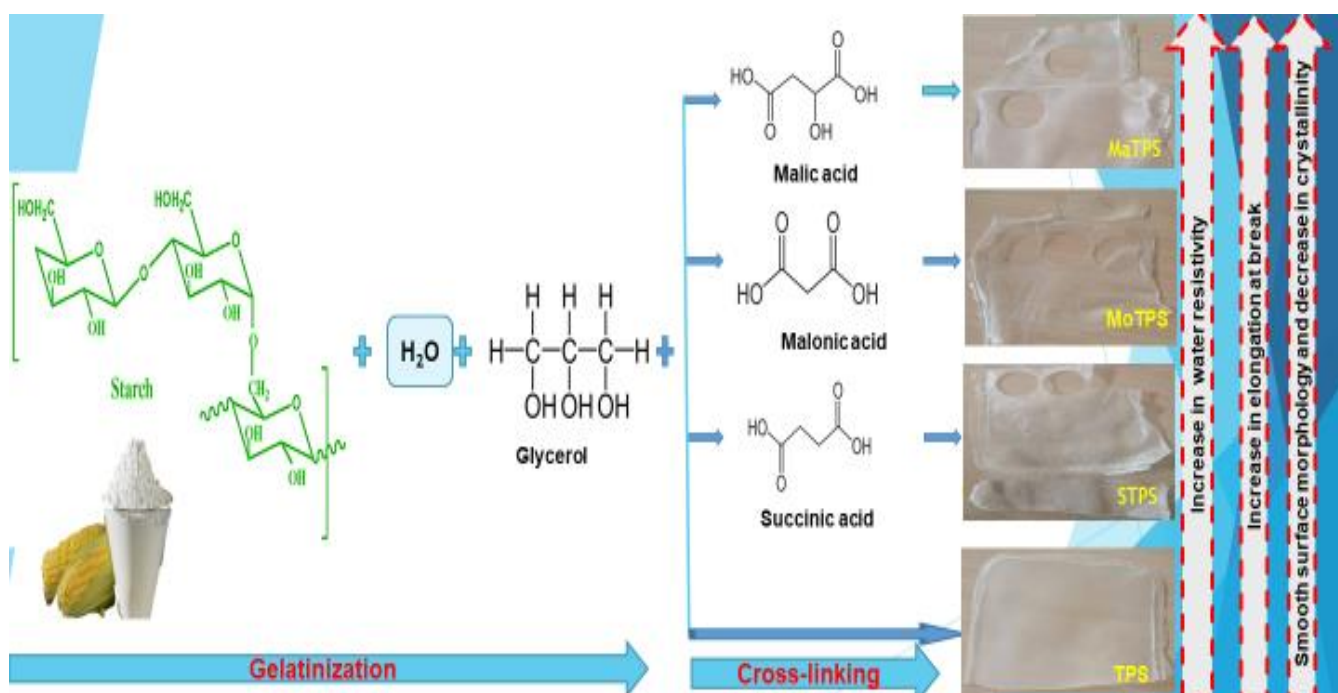


Figure A Effect of Acidity, number of hydroxyl (OH) groups per molecule, and carbon chain length ( $\text{CH}_2$ ) of carboxylic acids



### Thesis 3

Effects of number of carboxyl and hydroxyl functional groups; and solubility and other properties of carboxylic acids for crosslinking corn starch were examined. We investigated that the number of carboxyl functional groups in the carboxylic acids was found to have a more significant cross-linking impact. This could be because carboxyl groups can form stronger ester bonds with the hydroxyl groups of starch; than the hydrogen bonds that the hydroxyl groups can. Consequently, CTPS outperforms TaTPS, OTPS, and TPS in properties such as water resistance, tensile strength, and dimensional stability. The improved property of TaTPS over OTPS is also due to the increased number of hydroxyl groups in tartaric acid than oxalic acid.

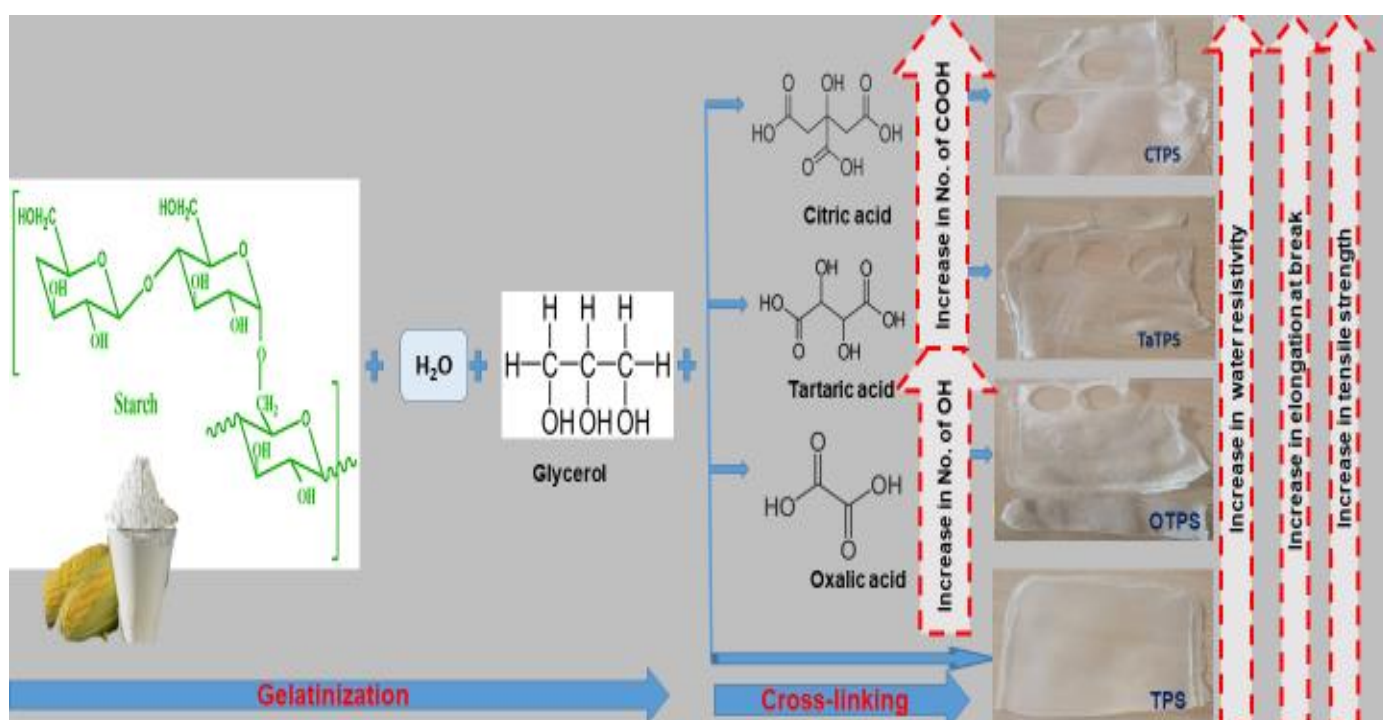


Figure B Effect of Number of carboxyl (COOH) and hydroxyl (OH) functional group of carboxylic acids

#### **Thesis 4**

Corn Starch film samples prepared using tartaric acid as cross-linker, sodium montmorillonite clay, termite clay (iron rich kaolinite) as fillers, cellulosic natural fiber (inset fiber) as reinforcement; and glycerol and water as plasticizers were characterized and tested. Both chemical and physical modification (crosslinking and reinforcing) techniques were used simultaneously. We explored that the inclusion of fillers don't hinder the crosslinking reaction. Tartaric acid can effectively crosslink corn starch even in the presence of fillers and improve the mechanical and water resistance property. Reinforcing of starch with cellulosic natural fibers and clay has significantly improved the strength and water absorption resistance.

#### **Thesis 5**

We prepared thin sheets of composite corn starch samples reinforced with pinewood fibre and calcium carbonate precipitate under a compression pressure and heating using a compression moulding machine. We investigated that the reinforcing starch using pinewood fibre provides better strength than reinforcing it with calcium carbonate precipitate and the hybrid composite. We also demonstrated that the hybrid composite has greater hardness and water absorption resistance followed by WTPS. This could be attributed to the better interfacial adhesion of the TPS matrix with pinewood fibre reinforcement. Moreover, we verified that WTPS exhibits better results in most of the measured proprieties given that TPS and wood fibre being both hydrophilic resulting in a homogeneous distribution and better interfacial adhesion.

#### **Thesis 6**

Corn starch was modified using terephthalic acid and Pyromellitic acid dianhydride under varying pH and ratio. We observed that unlike terephthalic acid; the Pyromellitic acid dianhydride has crosslinked corn starch. This could be due to the less number of carboxylic group per molecule and the very limited solubility of terephthalic acids compared to Pyromellitic acid dianhydride. As a result, the mechanical, water resistance and thermal property of the Pyromellitic acid crosslinked corn starch (PTPS) is significantly improved compared to TPS and TeTPS.

## List of Publications

### Journal Papers

1. Gerezgiher Alula Gebresas, Tamás Szabó, Kálmán Marossy, A comparative study of carboxylic acids on the cross-linking potential of corn starch films, *Journal of Molecular Structure*, Volume 1277, 2023, 134886, ISSN 0022-2860, <https://doi.org/10.1016/j.molstruc.2022.134886>.
2. Gerezgiher Alula Gebresas, Tamás Szabó, Kálmán Marossy, Effects of acidity, number of hydroxyl group, and carbon chain length of carboxylic acids on starch cross-linking, *Current Research in Green and Sustainable Chemistry*, Volume 6, 2023, 100354, ISSN 2666-0865, <https://doi.org/10.1016/j.crgsc.2022.100354>.
3. Gerezgiher Alula Gebresas, Tamas Szabó, Crosslinking of Starch Using Citric Acid. *IOP Conference Series: Materials Science and Engineering*, (2022, July). In *Journal of Physics: Conference Series* (Vol. 2315, No. 1, p. 012036). IOP Publishing <https://doi.org/10.1088/17426596/2315/1/012036>
4. Gerezgiher, A. G., Tamási, K., Ibrahim, J. F. M., Kónya, C., & Szabó, T. (2020, April). Thermoplastic corn starch reinforced with pine wood fibre and calcium carbonate precipitate filler. In *Journal of Physics: Conference Series* (Vol. 1527, No. 1, p. 012042). IOP Publishing. <https://doi.org/10.1088/1742-6596/1527/1/012042>
5. T.B. Yallew, E. Kassegn, S. Aregawi, A. Gebresas (2020, February). Study on effect of process parameters on tensile properties of compression molded natural fiber reinforced polymer composites. *SN Applied Sciences* (Vol. 2, No. 3, pages 1-8). Springer Nature Switzerland AG. <https://doi.org/10.1007/s42452-020-2101-0>
6. AG Gerezgiher, HA Bsrat, A Simon, T Szabó, (2019 March). Development and characterization of sisal fiber reinforced polypropylene composite materials. *International Journal of Engineering and Management Sciences* (Vo.4, No.1, p.348-358). University of Debrecen Publishing Platform. <https://doi.org/10.21791/IJEMS.2019.1.43>.

### Oral and Poster Presentation

1. Gerezgiher Alula Gebresas, Bsrat Halefom Aregay, Simon Andrea, Szabó Tamás, Development and characterization of sisal fiber reinforced polypropylene composite material for car door interior trim panel application, International Scientific Conference on Advances in Mechanical Engineering (ISCAME 2018), Debrecen, Hungary, 10/12/2018
2. Gerezgiher, Alula Gebresas, Flammability and chemical resistance of sisal fiber reinforced waste polypropylene composites, Antal Kerpely Doctoral School of Materials Science & Technology PhD forum, Miskolc, Hungary, 11/22/2018
3. Gerezgiher Alula Gebresas and Tamas Szabo, Property of Thermoplastic Corn Starch Reinforced With Natural Fiber and Filled With Calcium Carbonate Precipitate, International Scientific Conference on Advances in Mechanical Engineering, Debrecen, Hungary 7-9 Nov.2019
4. Gerezgiher Alula Gebresas and Tamas Szabo, Development and Characterization of Hybrid Biodegradable Polymer Composites, (ic-rmm4) 4<sup>th</sup> International Conference on Rheology and Modeling of Materials, Miskolc, Hungary, 7-10 Oct. 2019
5. AG Gerezgiher, K Tamási, JFM Ibrahim, C Kónya, T Szabó, Thermoplastic corn starch reinforced with pine wood fibre and calcium carbonate precipitate filler, Journal of Physics: IOP Conference Series (Vol. 1527, No. 1.), 1/20/2020, p. 012042
6. Gerezgiher Alula Gebresas and Tamas Szabo, Improving the Water Resistivity of Starch by Using Plasticization and Crosslinking as Modification Techniques, 9th Interdisciplinary Doctoral Conference (IDK2020), University of Pécs, Hungary, 27-28th of November 2020
7. Gerezgiher Alula Gebresas and Tamas Szabo, Modification of corn starch for improving the physical and mechanical properties for packaging purpose, Materials science day of PhD students, University of Panonia. 16, November, 2020
8. Gerezgiher Alula Gebresas, Tamas Szabo, Cross-Linking of Thermoplastic Starch For Improving Its Water Resistance And Physical Properties, MAS 14th International European Conference On Mathematics, Engineering, Natural and Medical Sciences, Széchenyi István University, Hungary, 3/26/2021
9. Gerezgiher Alula Gebresas, Tamas Tsabo, Modification of biodegradable Thermoplastic Corn starch for use in packaging, ic-cmtp6 the 6th International Conference on Competitive Materials and Technology Processes, Miskolc, Hungary, Oct/04/2021
10. Gerezgiher Alula Gebresas, TAMAS Szabo, Crosslinking of starch using citric acid, IOP conference series, Materials Science and Engineering, 3/3/2022

11. Gerezgiher Alula Gebresas, TAMAS Szabo, Crosslinking of starch using citric acid, International Scientific Research Conference, Van University, Turkey, 3/27/2022
12. Gerezgiher Alula Gebresas, TAMAS Szabo, Fabrication and Characterization of Corn Starch Films Modified by Acid Crosslinking, International Istanbul Congress, Istanbul, Turkey, 3/17/2022

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## Appendices

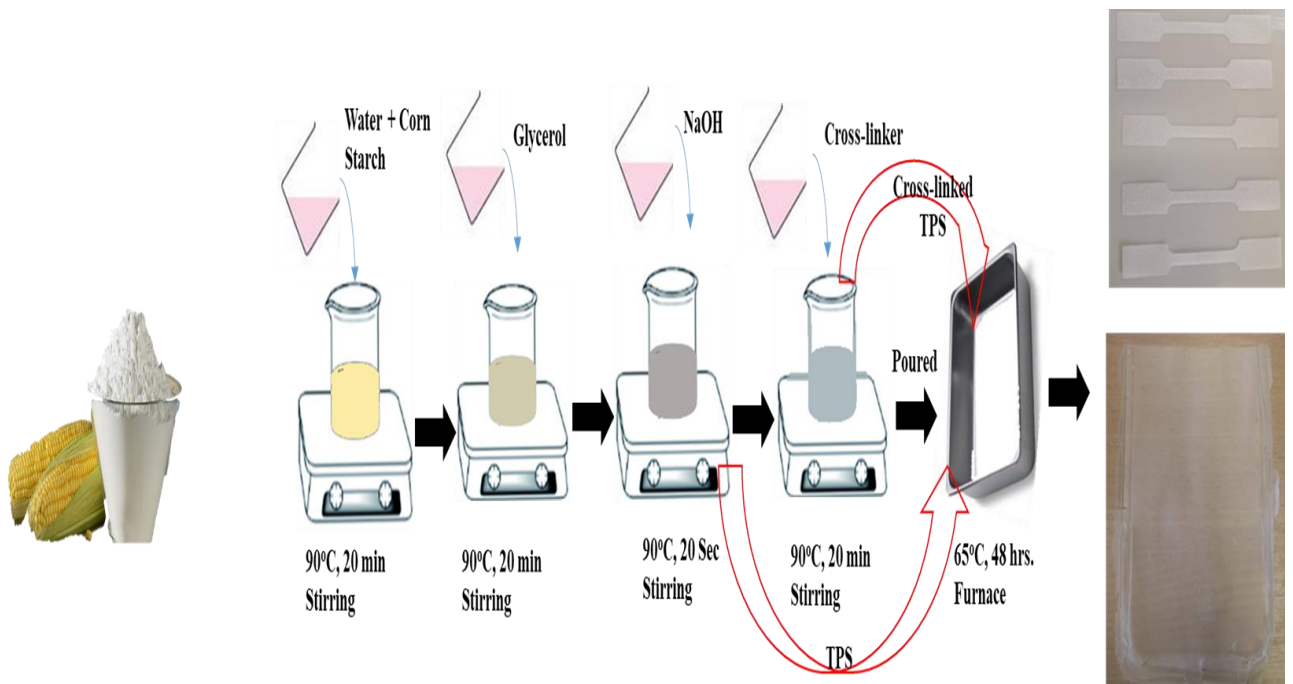


Figure A 1 Starch crosslinking flow chart

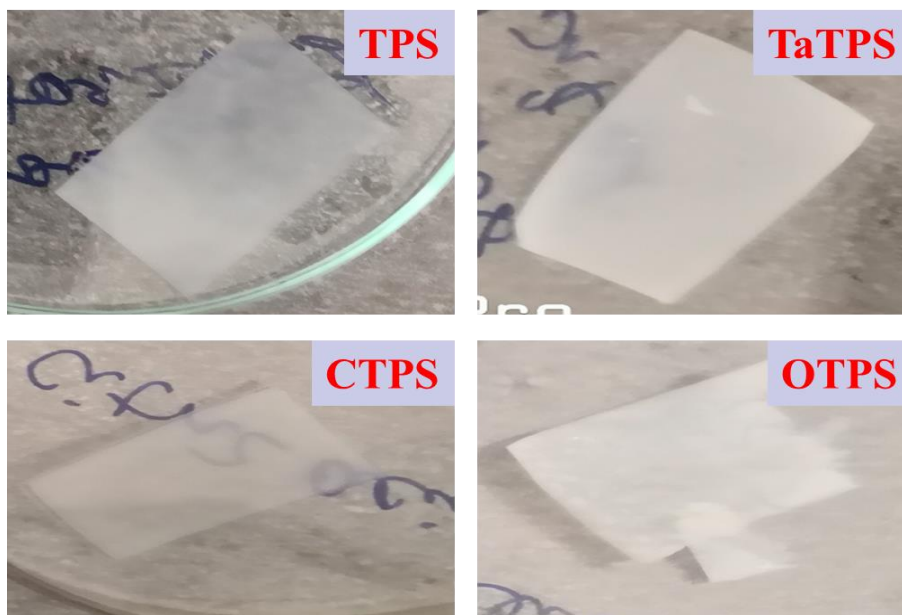


Figure A 2 Figure samples prepared using tartaric, citric and oxalic acids as modification agents.

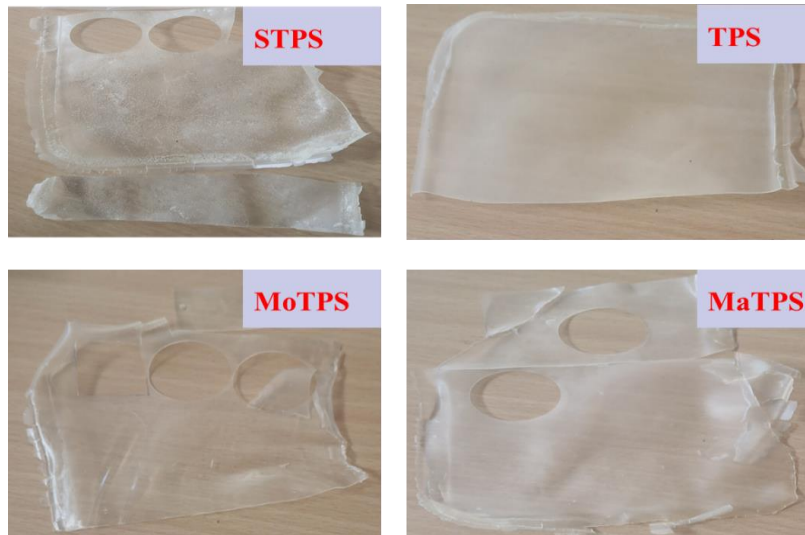


Figure A 3 samples prepared from succinic, malonic and malic acids as modification agents.

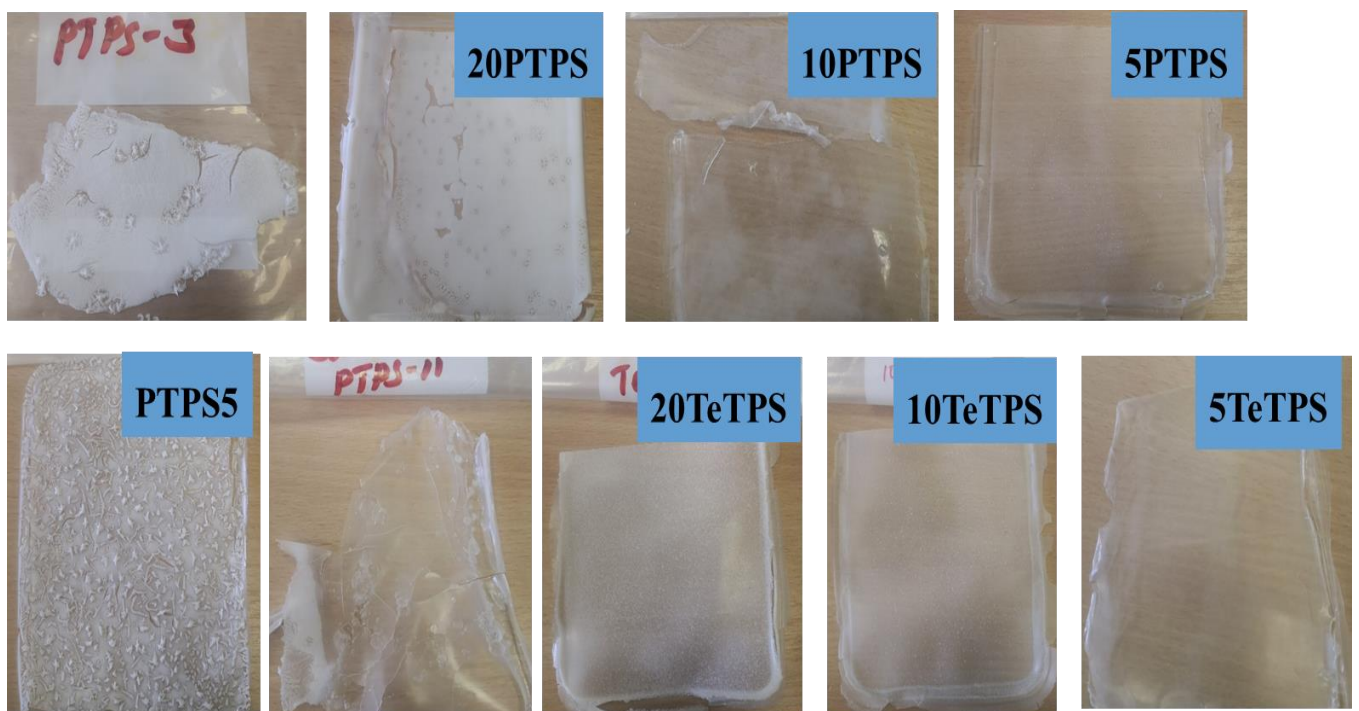


Figure A 4 samples prepared from terephthalic acid and pyromellitic dianhydride as modification agents.



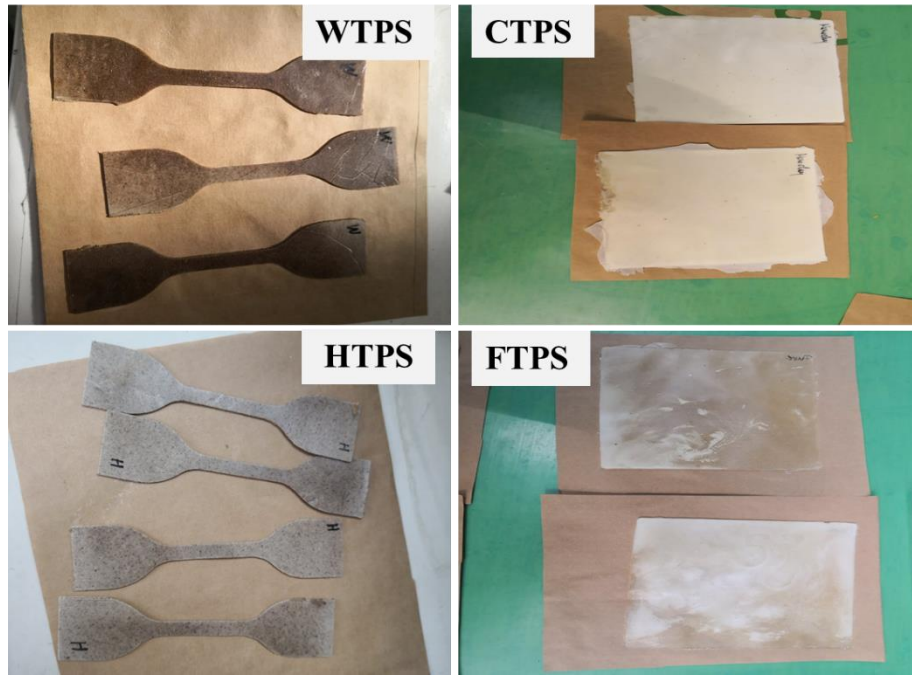


Figure A 5 composite samples prepared from pinewood fiber and calcium carbonate precipitate



Figure A 6 samples prepared from tartaric acid modified, cellulosic fiber and clay reinforced