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Thermal Stabilization of Polyhydroxyalkanoates by Washing with Acid

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Abstract

The main goal of this project was to investigate how the thermal stability of two different types of polyhydroxyalkanoates; poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) were affected when washing them with acid solution. Initially, four different acids were used: citric acid, hydrochloric acid, formic acid and acetic acid. Citric acid gave the best results for both biopolymers and was further investigated with respect to different concentrations and washing times to find the optimal parameters.
Experiments where deionized water was used as the washing solution, and experiments where citric acid was mixed into the samples were also investigated. The analyzing techniques used to evaluate the results was thermogravimetric analyze, differential scanning calorimetry, rheometer and size exclusion chromatography.

The degradation temperatures of as-received P(3HB) and P(3,4HB) powder were 279℃ and 247℃, respectively, determined with TGA. After the citric acid wash (1 mM, washing time 30 min), the degradation temperatures increased with 16℃ (296℃) for P(3HB) and with 48℃ (295℃) for P(3,4HB). Higher degradation temperatures could not be reached with higher concentrations or longer washing times. However, further investigation of P(3HB) showed that a concentration of only 0.05 mM and a washing time of 45 s was enough to raise the degradation temperature to 296℃. For P(3,4HB), a concentration of 1 mM and a washing time of 45 s was required to reach a degradation temperature of 295℃.

By washing P(3HB) with deionized water for 30 minutes, the degradation temperature became 294℃, and for P(3,4HB) it became 271℃. Due to the high increase in degradation temperature, more washing times were investigated. For P(3HB), a washing time of only 45 s was enough to raise the degradation temperature to 294℃, and for P(3,4HB) 5 min was required to reach 271℃.
When citric acid was mixed into the homopolymer, the degradation temperature decreased with 27℃, and for the copolymer the degradation temperature stayed the same.
The increase in degradation temperature by washing the biopolymer powder indicates a significant increase in the thermal stability.

Sammanfattning

Målet med detta projekt var att undersöka hur den termiska stabiliteten hos två olika polyhydroxialkanoater; poly(3-hydroxibutyrat) och poly(3-hydroxibutyrat-*co*-4-hydroxibutyrat) påverkades genom att tvätta dem med syra. Ursprungligen undersöktes fyra olika syror: citronsyra, saltsyra, myrsyra och ättiksyra. Citronsyra gav de bästa resultaten för båda biopolymererna och undersöktes med olika koncentrationer och tvättider.
Experiment där avjonat vatten användes som tvättlösning och experiment där syra blandades in i provet genomfördes också. Den analysutrustning som användes för att utvärdera resultaten var thermogravimetric analyze, differential scanning calorimetry, reometer och size exclusion chromatography.

Nedbrytningstemperaturerna för obehandlat P(3HB) och P(3,4HB) var 279℃ och 247℃, vilket bestämdes med TGA. Efter citronsyratvätten (koncentration på 1 mM, tvättid 30 min) höjdes nedbrytningstemperaturen med 16℃ (296℃) för P(3HB) och med 48℃ (295℃) för P(3,4HB). Högre nedbrytningstemperaturer kunde inte nås med ökad syrakoncentration eller längre tvättider. Vidare undersökning av P(3HB) visade dock att en koncentration av endast 0.05 mM och en tvättid på 45 s räckte för att höja nedbrytningstemperaturen till 296℃. För P(3,4HB) krävdes en koncentration på 1 mM och en tvättid på 45 s för att nå en nedbrytningstemperatur på 295℃.

Genom att tvätta P(3HB) med vatten i 30 minuter blev nedbrytningstemperaturen 294℃ och för P(3,4HB) blev den 271℃. På grund av denna höga temperaturhöjning undersöktes fler tvättider. Det visade sig att en tvättid på endast 45 s räckte för P(3HB) för att nå nedbrytningstemperaturen på 294℃, och för P(3,4HB) krävdes 5 min för att nå 271℃. När citronsyra blandades med homopolymerpulvret sjönk nedbrytningstemperaturen med 27℃, medan nedbrytningstemperaturen förblev den samma för sampolymeren.
Höjningen av nedbrytningstemperaturen som fås genom att tvätta biopolymerpulvret tyder på en signifikant ökning av den termiska stabiliteten.

Preface

This master thesis has been carried out together with the center for Analysis and Synthesis (CAS) at the department of chemistry in Lund, from November 2016 to March 2017. All laboratory work and analysis were performed at CAS.

A special thanks to Carlos Arza Rodriques for helping us to plan our work, explaining to us how the analyzing equipment worked, and showing large interest in our findings.

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Table of contents

1 Introduction 1
  1.1 Background 1
  1.2 Aim 1
  1.3 Approach 1
2 Theory 2
  2.1 Polyhydroxyalkanoates 2
  2.2 Previous Studies with the Aim of Increasing the Thermal Stability of PHA 4
  2.3 Acids and Parameters to Study 6
    2.3.1 Acids Selected for the Experiment 6
    2.3.2 Facts About the Acids 7
    2.3.3 Parameters to Study 7
  2.4 Analyzing Equipment 8
    2.4.1 Rheometer 8
    2.4.2 Size Exclusion Chromatography (SEC) 9
    2.4.3 Thermogravimetric Analysis (TGA) 10
    2.4.4 Differential Scanning Calorimetry (DSC) 11
3 Experimental Part 13
  3.1 Materials 13
  3.2 Method 13
    3.2.1 Washing, Filtration and Drying 13
    3.2.2 Analyzing Methods 15
  3.3 Eight Experimental Parts 18
    3.3.1 Experimental Overview 18
    3.3.2 Part 1 – Four Different Acids 21
    3.3.3 Part 2 – Citric Acid, Different Concentration 21
    3.3.4 Part 3 – Citric Acid, Washing Times 22
    3.3.5 Part 4 – Comparing Unprocessed and Processed Samples 22
    3.3.6 Part 5 – Formic Acid, Different Concentrations 23
    3.3.7 Part 6 – As-received Powder with Citric Acid 23
    3.3.8 Part 7 – Water Wash 23
    3.3.9 Part 8 – Error Sources 24
4 Results and Discussion 25
  4.1 Important Results Obtained from Each Method 25
    4.1.1 TGA 25
    4.1.2 Rheometer 25

4.1.3 SEC 25
4.1.4 DSC 25
4.2 Part 1 – Four Different Acids 26
4.2.1 P(3HB) 26
4.2.2 P(3,4HB) 28
4.2.3 Comparing TGA and Rheometer Results 30
4.3 Part 2 – Citric Acid, Different Concentrations 30
4.3.1 P(3HB) 30
4.3.2 P(3,4HB) 34
4.4 Part 3 – Citric Acid, Washing Time 38
4.4.1 P(3HB) 38
4.4.2 P(3,4HB) 41
4.5 Part 4 – Comparing Unprocessed and Processed Samples 44
4.5.1 P(3HB) 44
4.5.2 P(3,4HB) 46
4.6 Part 5 – Formic Acid 48
4.6.1 P(3HB) 48
4.6.2 P(3,4HB) 49
4.6.3 Common conclusion 49
4.7 Part 6 – As-received Powder mixed with Citric Acid 49
4.7.1 P(3HB) 49
4.7.2 P(3,4HB) 50
4.8 Part 7 – Water Wash 51
4.8.1 P(3HB) 51
4.8.2 P(3,4HB) 52
4.9 Part 8 – Error Sources 53
4.9.1 SEC 53
4.9.2 DSC 55
5 Conclusion 57
5.1 Future work 57
6 References 58
7 Appendix 60
7.1 Appendix 1 60
7.2 Appendix 2 61
7.3 Appendix 3 63

1 Introduction

1.1 Motivation

Today, plastic materials play a very important role in our everyday life. Their physical properties make them very useful in many different areas. The major disadvantage with these plastics, made by fossil sources, is that they are non-degradable and thus, they accumulate in the environment at a rate of 25 million tons per year. Because of this, there is a strong need for a new polymer with degradable properties to replace the well-established plastic.

One group of biopolymers that incorporate many of the desired properties is polyhydroxyalkanoates (PHA). This biopolymer is a potential alternative to plastics made from non-degradable sources. Its major disadvantage is that it is thermally unstable when processed. To be able to use PHA in the industry as a replacement for fossil based plastics, a solution to the thermal instability needs to be found [1].

1.2 Aim

The aim of this thesis was to find an acid and parameters, such as time and concentration, to produce the most thermally stable biopolymer. Two different polymers were studied; one homopolymer, poly(3-hydroxybutyrate) [P(3HB)] and one copolymer, poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3,4HB)]. Four different acids were selected to establish pK_a, structure, molecular weight and price range. They were then further experimentally investigated. The goal of the experimental part was to examine how these acids, with different parameters, would affect the properties of the biopolymer by washing.

1.3 Plan

1. Make a literature survey to find out what has been done in this area.
2. Continue the literature survey to include facts about the different potential acids and determine; washing time and solution concentration for the experiment. The literature survey will be continuously upgraded with relevant information as the experimental work goes on.
3. Perform all the experiments and continuously evaluate the results.
4. Write the final report
5. Oral presentation

2 Theory

2.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are biobased, biodegradable, and sustainable polyesters. They are the most studied family of biopolymers today as a potential alternative to petroleum-based plastic due to their unique production by bacterial fermentation of for example waste water, cellulose and starch [2]. After the fermentation step, the bacterial PHA is pre-treated, extracted and purified by using, in most cases chloroform and a low molecular weight alcohol [1]. One major remaining disadvantage of the PHA is that they are thermally unstable due to destabilizing impurities, for example, metal ions. When PHA reaches temperatures close to its melting point, thermal decomposition begins by chain scission through β-elimination (Figure 1). The chains break down into smaller sub-chains, and crotonic and carboxylic acid end groups are generated because of the β-elimination reaction. This results in an alternation of the mechanical properties for the biopolymer due to the decrease in molar mass.

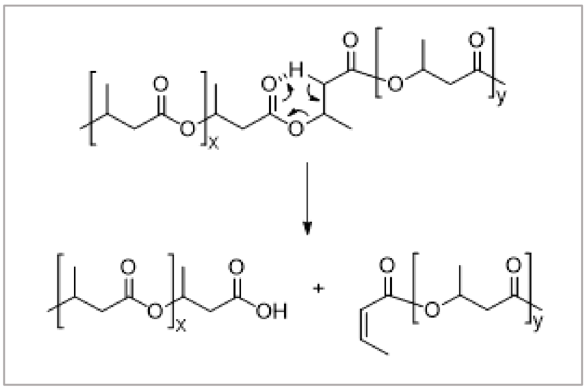


Figure 1. The result of β-elimination when a chain is subjected to thermal decomposition as a result of too high temperatures.

There are many different types of PHA, the most common being poly(3-hydroxybutyrate) (P(3HB)). P(3HB) was the first type of PHA to be studied, characterized and commercialized. Its success in the biopolymer industry is because of its mechanical properties who is like those of well-established polymers such as polypropylene. The physical properties of PHA depend on the chemical structure of the repeating unit [3].

P(3HB) has a high crystallinity and a melting point of 180 °C. The high crystallinity leads to a brittle material and processing difficulties. To overcome this drawback that limits its application, a copolymer such as poly(3-hydroxybutyrate-co-4-hydroxybutyrate) can be manufactured. Both the melt temperature and the crystallinity degree is lower for P(3,4HB) compared to P(3HB). This results in a higher flexibility and an improved thermal stability for the copolymer. Unfortunately, P(3,4HB) has some disadvantages as well, such as slow crystallization rate and weak mechanical properties [4]. A wide range of compositions can be produced, all from 0 to 100 mol% 4HB unit. The different copolymers produced generate everything from rigid thermoplastics to elastic rubber materials. If the 4HB content exceeds 25% the copolymer becomes amorphous. With an increasing 4HB content both the crystallization rate and the degree of crystallization decreases.

One article studied the crystallization behavior of different P(3,4HB) blends [5]. The blends consisted of two different P(3,4HB). One of the polymers had a content of 33 mol% 4HB and was amorphous. The other polymer was semicrystalline and had a content of 11 mol% 4HB. The different mass ratios evaluated (P(3HB-co-11%-4HB)/ P(3HB-co-33%-4HB)) were 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90 and 0/100. The results from the DSC can be seen in Figure 2 below [5].

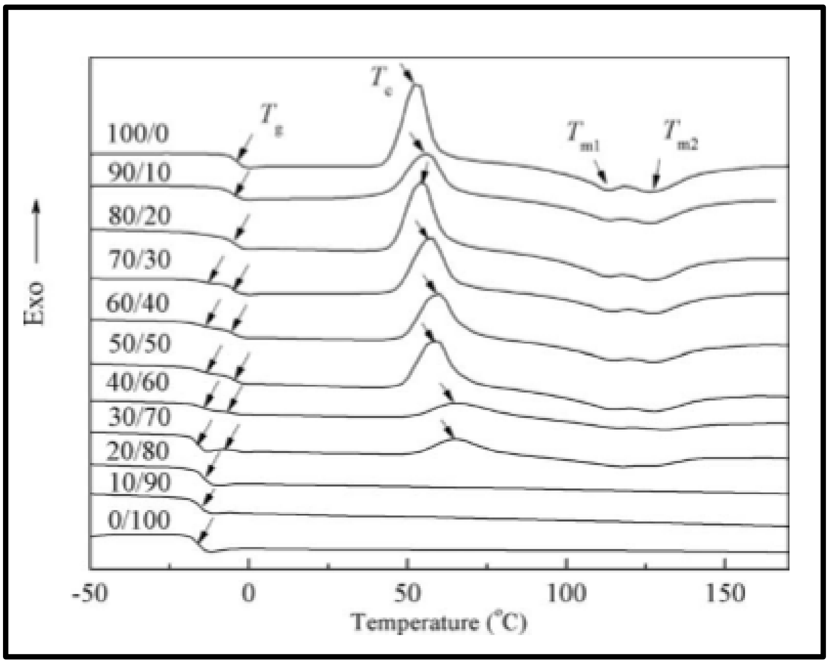


Figure 2. DSC thermograms of the different blends from the second heating run with a heating rate of 5 ℃/min [5]. The blends vary from 0 – 100 % 4HB present and as a result, the temperatures are affected.

As can be seen in Figure 2, the properties of the samples are changed a lot when the fraction between the different polymers are being varied. 100/0 has a clear crystallization peak and two melting peaks, while 0/100 shows only a glass transition temperature. The amorphous part in the sample is successively increased which leads to this result.

The structures of P(3HB), P(4HB) and P(3,4HB) can be seen in Figure 3-5.

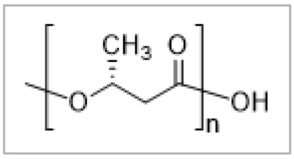


Figure 3. The structure of P(3HB).

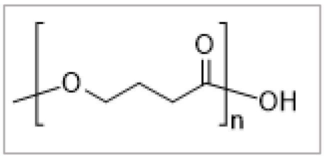


Figure 4. The structure of P(4HB).

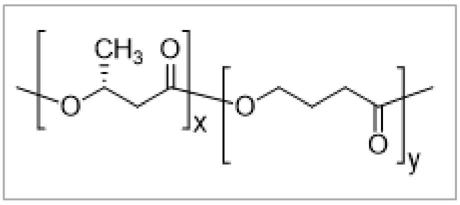


Figure 5. The structure of P(3,4HB), which is Figure 3 and 4 combined.

2.2 Previous Studies with the Aim of Increasing the Thermal Stability of PHA

When searching for information about washing PHA with acid to improve the thermal stability, one concludes that this research area is quite unexplored. Many scientific articles describe the pre-treatment, extraction and purification of PHA after its formation by bacterial fermentation [6]. Further information on how to avoid thermal decomposition when PHA is heated is hard to find.

There is however some articles describing the addition of different types of property modifiers, such as thermal stabilizing agents, anti-oxidants, plasticizers, and processing aids [6]. One article describes the addition of tannic acid (TA), a naturally occurring polyphenol, which improves the processing and use performance of P(3HB), while keeping its biodegradable attitude [6]. The stabilization is the result of crosslinking between TA and P(3HB). The effect is a lowered and delayed thermal degradation process. The TGA analysis in Figure 6 shows the shift of the weight loss curve to higher temperatures [6].

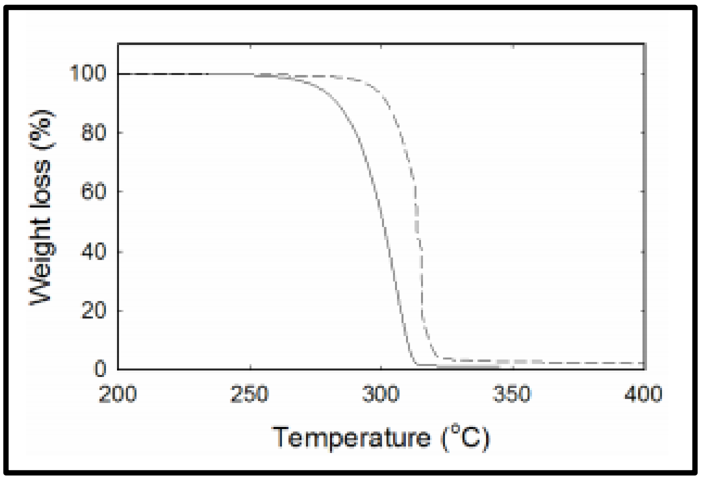


Figure 6. TGA analysis of the neat P(3HB) (solid line) and of the PHB/TA15 compound (dashed line), with the weight loss percentage as a function of temperature [6].

Another scientific article that studied the use of additives has investigated the presence of low molecular weight propylene glycol (LMWPPG), among others, blended with P(3HB) [7]. The intramolecular interaction of LMWPPG with P(3HB) chains hinders the mechanism of the β-elimination that causes chain scission and thermal degradation. The addition of LMWPPG results in a decrease in the crystallinity and on the melt temperature with a simultaneous increase on the decomposition temperature. The processing window is significantly increased from 105 to 134℃. The results from a TGA analysis can be seen in Figure 7 [7].

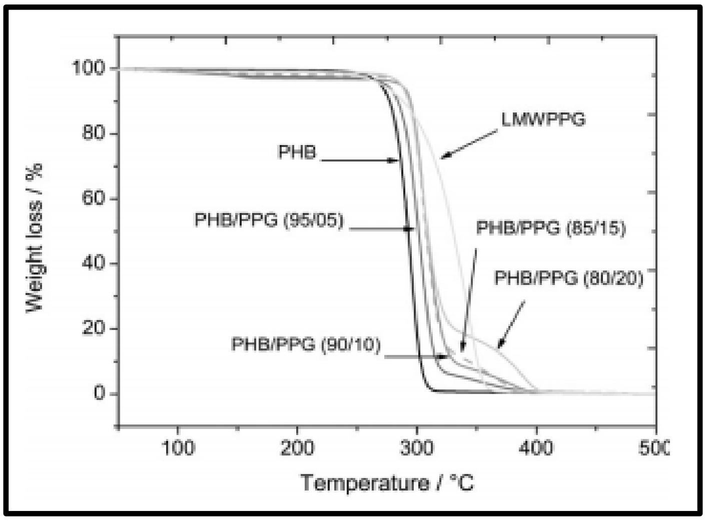


Figure 7. TGA analysis of the neat PHB and of the PHB/LMWPPG blend, with the weight loss percentage as a function over temperature [7].

One scientific article described how the thermal stability was increased by washing PHA with acid [8]. The PHA chosen for the experiment was P(3,4HB) and the acid used was hydrochloric acid (HCl). The P(3,4HB) was first washed in a 1 mM HCl solution. This was done in room temperature for 30 minutes. P(3,4HB) was then washed three times with deionized water, before filtered and dried at 50℃ for 24 h under vacuum. The results shown from the TGA analysis are similar to those when additives were used. The decomposition temperature increased by up to 50℃, see Figure 8.

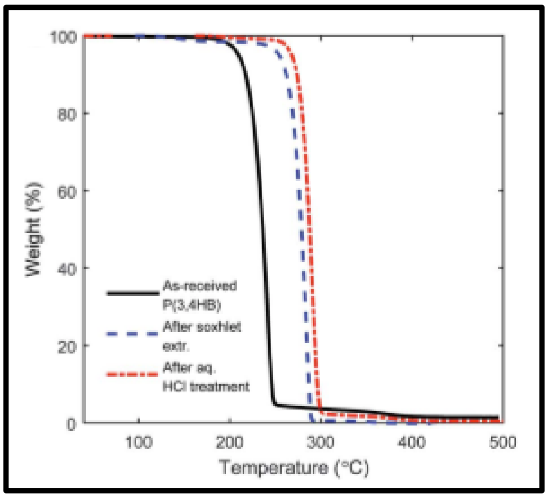


Figure 8. TGA analysis of as-received P(3,4HB), and P(3,4HB) after HCl treatment, with the weight loss percentage as a function of temperature [8].

The same article also showed the TGA result of the weight loss percentage as a function of time, with a remarkable improvement of the thermal stability. Here, both P(3HB) and P(3,4HB) were tested. For P(3HB) at 180℃, the weight loss percentage after 1 h was less than 1 wt% for both the as-received and acid-treated P(3HB). After 8 h it was a significant difference between the samples. The as-received sample had lost 99 wt% while the acid-treated sample only lost 10 wt%.

The result from the P(3,4HB) at 160℃ was even more remarkable. After 1 h, the weight loss percentage for the as-received sample was 10 wt% while for the acid-treated sample it was below 1 wt%. After 8 h, the corresponding weight loss percentage was 95 wt% and 1 wt%. The weight loss percentage for the acid-treated sample had barely changed, see Figure 9 [8]. This is one of the reasons why a further investigation of acid washed PHA is being made in this thesis.

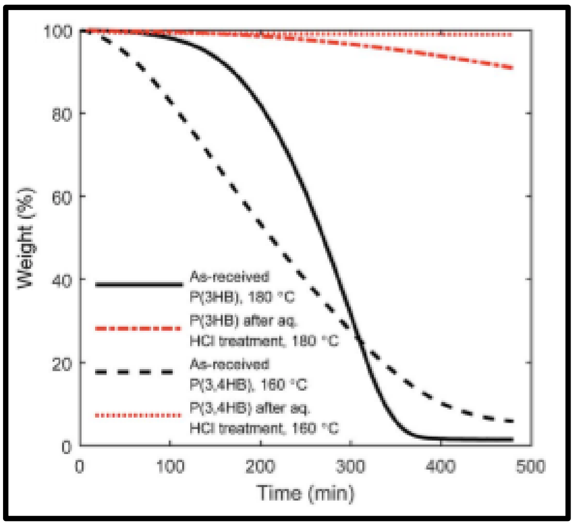
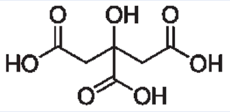
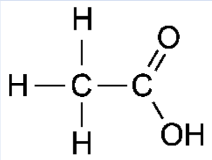
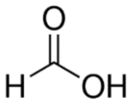


Figure 9. TGA analysis of as-received P(3,4HB) and P(3HB), and P(3,4HB) and P(3HB) after aqueous HCl treatment, with the weight loss percentage as a function over time [8].

2.3 Acids and Parameters to Study

Since not much information about previous surveys how to increase the thermal stability by acid wash have been found, it was hard to know what specific properties the ultimate acid should have. Therefore, an investigation of acids that contains different physical properties was made before the experimental part took place. Table 1 shows the structure, molecular weight, pK_a and price for the four different acids that were chosen [9], [10].

Table 1. A comparison is made between four different acids; citric acid, hydrochloric acid, acetic acid and formic acid. The properties; structure [9], molecular weight [9], pK_a [9], and price [10] is displayed in the table.

Acid	Structure	Molecular weight (g/mole)	pK_a	Price
Citric acid		192.12	3.13 (1) 4.76 (2) 6.40 (3)	185 kr / 500 g
Hydrochloric acid	H−Cl	36.46	-6.3	41 kr / 1 l
Acetic acid		60.05	4.76	53 kr / 1 l
Formic acid		46.03	3.77	243 kr / 1 l

2.3.1 Acids Selected for the Experiment

Four acids were selected for further experimental investigation.

Hydrochloric acid was chosen because it is inorganic. It is a strong acid and it showed very promising results when being used as a pre-treatment agent for biopolymers in [8].

Citric acid works as a chelating agent which means that it encloses the metal ion. The washing result of such an acid would be interesting to find out. The citric acid also has a high molecular weight compared to the other acids.

Acetic acid and formic acid were chosen because they show similar properties. They are both organic and aliphatic, and their molecular weights are in the same range. They were chosen in order to investigate if two similar acids would yield the same result or not.

2.3.2 Facts About the Acids

2.3.2.1 Citric acid

Citric acid is a weak, organic tricarboxylic acid. It is colorless, odorless, and have a relatively strong acid taste. Citric acid is found in citrus fruits in large quantities, from 5%. One may also find it in almost all animal species and plants. It is widely used for flavoring, as an acidifier and as a chelating agent. A chelating agent, also called a chelant, is a molecule that contains at least two electron donor atoms that can bind to a single metal ion. During this binding, there is a ring created with the metal ion in the middle. This cyclic structure that is formed is called a chelation complex [11]. The acid is also quite known for its so called Citric acid cycle. It is pivotal in the oxidation of sugars and acetates to carbon dioxide and water, releasing energy for physiological functions [12].

2.3.2.2 Hydrochloric acid

Hydrochloric acid is an inorganic acid, also known as muriatic acid, to indicate the presence of chlorine in an inorganic compound. Hydrogen chloride can be found naturally in gases evolved from volcanoes. This due to its formation when high temperature reaction occurs between water and salts found in seawater. It is also present in the atmosphere and in the digestive system of most mammals. Hydrogen chloride is produced by the direct reaction of hydrogen and chloride, by reaction of metal chlorides and acids, and as a by-product from many chemical manufacturing processes such as chlorinated hydrocarbons [13].

2.3.2.3 Acetic acid

Acetic acid is a clear and colorless liquid, but with a sharp odor, burning taste, and blistering properties. The acid is found in dilute solutions in many plants and animals. It is also found in ocean water, oilfield brines and rain. Vinegar, produced by fermentation of wine, contains about 4-12% acetic acid. It can be used for various applications. More than 65% goes into polymers derived from vinyl acetate or cellulose. Most of the poly (vinyl acetate) is used in paint, coatings and plastics [14], [15].

2.3.2.4 Formic acid

Formic acid, also known as methanoic acid, is the first member of the homologous series of alkyl carboxylic acids. It is a corrosive, colorless liquid with a strong odor. The defensive secretion of several insects, ants in particular, contains formic acid. The acid was first isolated from the formic acid produced by ants in 1671, until 1856 when preparation of the acid with a laboratory method was discovered. The production of formic acid was modest until 1960s when it became available as a by-product of the production of acetic acid by liquid-phase oxidation of hydrocarbons [16].

2.3.3 Parameters to Study

There was two different parameters that were of interest to study along with the type of acid. These were acid solution concentration and washing time.

2.3.3.1 Concentration

One interesting aspect was to find out how the purity of the biopolymer was affected by different concentrations of the acids. From an economically point of view, an acid solution with a low concentration is favorable.

2.3.3.2 Time

The purity of the PHA after washing with acid might also depend on the time the sample was washed. Therefore, different washing times were performed to see if time has any effect. A time as short as possible would increase the efficiency.

2.4 Measurement techniques

2.4.1 Rheometer

A rheometer is used to study and analyze the dynamic mechanical properties of polymers in the melt state. It can detect thermal transitions of polymeric materials.
In a rheometer, the studied material is subjected to a sinusoidal strain or stress at varying temperatures and frequencies. Depending on the properties of the material, the response of such as molecular mobility and degree of crystallinity will differ, and it can be determined whether the material is elastic, viscoelastic or viscous. If a material is 100% elastic, the stress and strain curves will be in phase with each other (all the energy is stored). If the curves instead differ with 90°, the material is found to be 100% viscous/liquid (all the energy is lost by heat) [17]. If a value between 0 and 90° is obtained, then the material is visco-elastic. Most polymers show a visco-elastic behavior, this means that they undergo both irreversible ("loss of" energy, viscous flow) and reversible ("store of" energy, elasticity) changes [18]. A diagram with stress and strain amplitudes of a viscoelastic material can be seen in Figure 10.

In principe, a rheometer measures how a material responds to an applied force. Four important parameters can be measured; modulus, phase angle, damping and time depending visco-elastic properties. The modulus describes the stiffness of the material, and how well the material can resist deformation. The damping explains the capacity to absorb energy. Viscoelastic materials exhibit both elastic and damping behavior and it is the damping that causes the strain to be out of phase with the stress during a measurement. The dynamic shear modulus (G_d) consists of one shear storage modulus (G') and one shear loss modulus (G''). G' is a measure of the materials elasticity. When a polymer is in its glassy state the value of G' is high, but when the temperature is raised, the material becomes more soft and rubbery and the value decreases. Figure 11 shows how the storage modulus is changed with temperature, which depends on the properties of the polymer. G'', the loss modulus is associated with viscous energy dissipation such as damping [17].

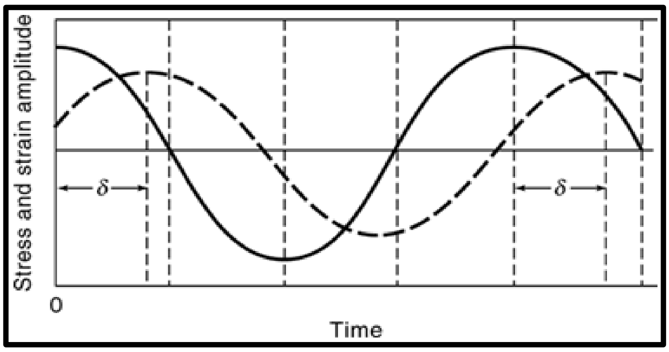


Figure 10. Shows a viscoelastic material, stress (---) and strain (- - -) amplitudes vs time. δ is the phase angle that defines the lag of the strain behind the stress [17].

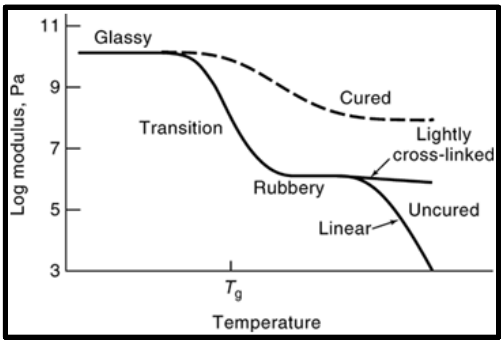


Figure 11. Shows how the modulus for a polymeric material is changed with increased temperature [17].

2.4.2 Size Exclusion Chromatography (SEC)

The molecular weight or molecular size plays an important role in determining properties of polymeric materials. Some of the properties that are affected by the molecular size are; process ability, toughness, glass-transition temperature, hardness and melt viscosity.
Most synthetic polymer samples are composed of up to thousands of chains with different molecular weights which results in characteristic molecular weight distributions. An example of a polymers molecular weight distribution can be seen in Figure 12. The shape and width of this distribution will depend largely on the polymerization mechanism and conditions.

SEC is a commonly used characterization method for polymers because of its capability to provide molar mass distributions with good results. By using a size exclusion chromatograph, the number average M_n and weight average M_w can be determined. The width of the distribution is calculated from the ratio of M_w/M_n and is called the polydispersity (PDI).
SEC separates the polymer chains based on molecular hydrodynamic size. To perform SEC, the polymer must be dissolved in a suitable solvent. The polymer solution is then injected into a column that consists of packed porous particles. These particles have a quite defined particle size. The mobile phase in the SEC is often the same solvent that is used to dissolve the polymer. As the polymer is transferred through the column, small molecules will penetrate the pores, as they are small enough to enter. The smaller the molecules are, the longer the retention time in the column is. Larger molecules are too big to enter the pores and is instead just passing by the pores in the column without entering them. Since the small molecules can enter the pores and diffuse freely in and out, they will elute later than the larger molecules. First out of the SEC are high molecular weight components followed by low molecular weight components.
To get reliable results from the measurements, the SEC must be calibrated with a polymer standard that has a known molecular weight, before the specific analyze can be carried out.

A SEC can mostly separate polymer chains with weights between 100 and 1 000 000 g/mole, which means that both small molecules and high molecular weight molecules can be separated depending on the pore size of the packing material. There are two important parameters that must be considered before examining the measurement; the sample must be able to dissolve and there can be no enthalpy interactions present between the packing material and the sample [19].

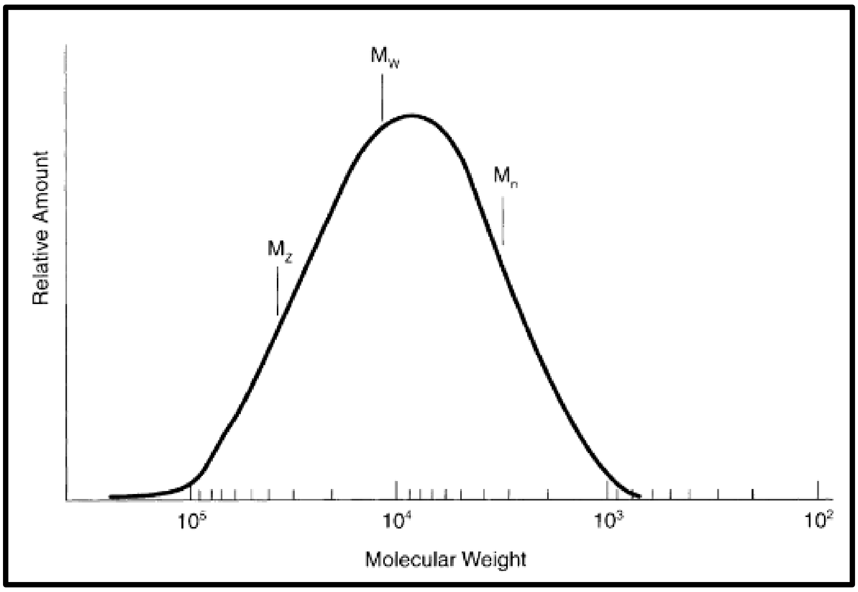


Figure 12. Molecular weight distribution of a polymer showing the locations of the number-, weight- and z-average molecular weights [19].

2.4.3 Thermogravimetric Analysis (TGA)

Thermogravimetry (TG) is a method that is used to measure changes in mass of a sample as a function of increased temperature (the heating rate is constant) or time (the temperature is constant). There are a lot of processes that can be studied by using this type of analyzing method. Adsorption or absorption will show a weight gain during the heating, while for example desorption, vaporization and decomposition will show a weight loss.

To perform these kinds of measurements a thermobalance is used. A schematic arrangement of all components included in the setup can be seen in Figure 13 below.

The balance works in such a way that it transmits a continuous measure of the sample mass to a recording system such as a computer. The computer creates a TG curve with sample mass on the y-axis and temperature or time on the x-axis. An example of TG curves for four different polymers can be seen in Figure 14.

The heating rates usually range from 5 – 20 K/min although other special setups can heat a sample much faster. The most common pressure in the balance is atmospheric pressure. To be able to use a vacuum atmosphere, special equipment is required. The arrangement of the different components in the apparatus may vary a lot. For example, the furnace may have different locations according to the balance (below, above or in line with). The container that holds the sample is often cylindrical but flat plates is also an option [20].

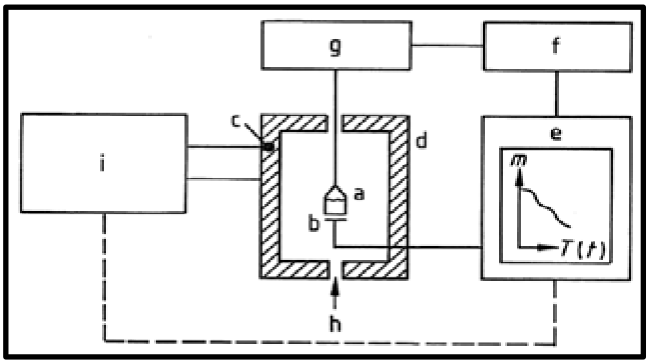


Figure 13. Schematic picture of a thermo balance a) Sample; b) Temperature sensor (sample); c) Temperature sensor (furnace); d) Furnace e) Computer logging temperature, time and mass of the sample; f) Balance controller; g) Recording microbalance; h) Gas; i) Furnace temperature programmer [20].

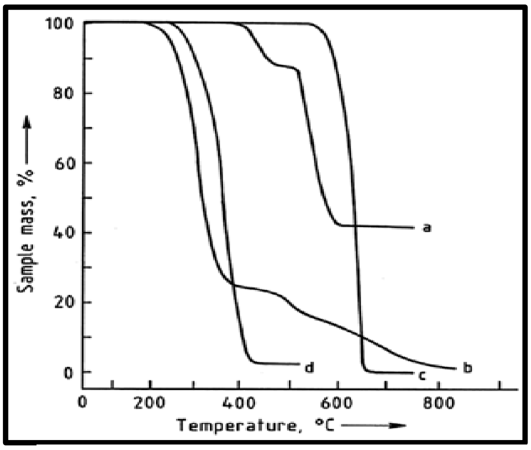


Figure 14. TG curves showing the relative stabilities of four different polymers in air, a) Silicone rubber; b) PVC; c) PTFE; d) Perspex [20].

2.4.4 Differential Scanning Calorimetry (DSC)

A DSC is based on the measurement of heat changes. It is used to study phase transitions and chemical reactions in a material when it's heated, held at a constant temperature, or cooled. A DSC can also be used to determine the crystallinity of a polymer sample.

In the measuring instrument its room for one sample pan and one reference pan. A common material for the pan is aluminium. A schematic picture of DSC equipment can be seen in Figure 15. During the DSC measurement, the temperature for the system is raised and the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature or time. The temperature difference, for an almost perfectly designed instrument, remains constant during the whole measurement if the sample does not undergo any transition and no reactions are taking place.

Crystallization and melting are transitions that are of the first order. This will show as a peak in the DSC-curve. When for example melting occurs in the sample, this transition requires heat and is endothermic, therefore the temperature difference between the sample and the reference is lower than before the transition takes place, which will result in a negative peak, with a minimum, in the created DSC curve. For exothermic transitions or reactions, such as crystallization, the peak is then in the opposite direction, showing a maximum. This because the temperature difference between the sample and the reference is higher than if no reaction or transition was taking place. When crystallization takes place, new bonds are being formed which leads to an exothermic transition. The area under the peak is directly proportional to the energy that is emitted or absorbed during the transformation in the sample.

Polymers often go through a glass transition during heating. The glass transition temperature is described as the temperature where the material changes from a hard, brittle state into a molten or rubbery state. The glass transition is of the second order and will show as a lowering/a step of the DSC curve. In semi crystalline polymers, the glass transition can be hard to find. Another explanation to not be able to determine the glass transition is if the difference in heat capacity is not big enough for the equipment to detect.

DSC is a method that is widely used to study polymers. A DSC curve for a polymer is showed in Figure 16 below.

The crystallization will show as a positive peak in the curve, as this is an exothermic reaction. Followed by the crystallization is the melting of the polymer, which requires heat and is shown as a negative peak in the curve [20].

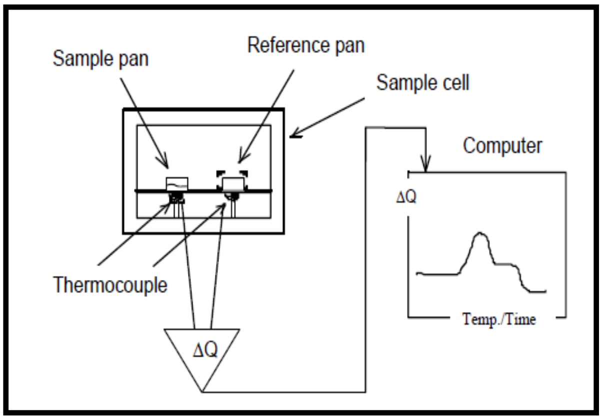


Figure 15. Schematic picture of a DSC equipment with the thermocouple, sample pan, reference pan, sample cell, and computer market out [20].

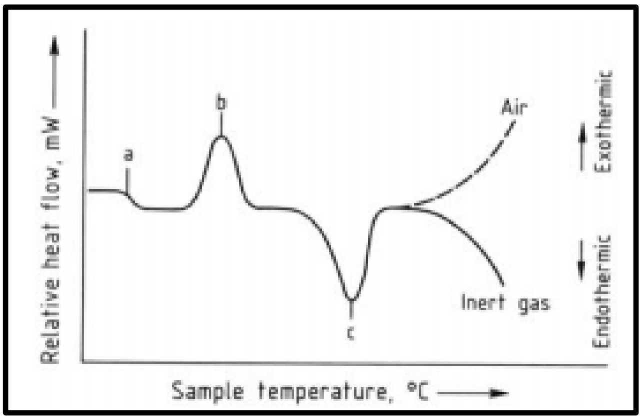


Figure 16. DSC curve for a polymer showing the glass transition temperature (a), the crystallization temperature (b), and the melting temperature (c) [20].

2.4.4.1 Parameters

Important parameters obtained from DSC were degree of crystallinity, crystallinity temperature, melting temperature and glass transition temperature. How the molar mass affected these parameters is described below.

2.4.4.1.1 Crystallinity and crystallization

The crystallinity is an important property of the polymer. To calculate the crystallinity degree (X_c) of a P(3HB) sample from DCS results, the following equation was used:

$$X_c\% = 100 \cdot \left(\frac{\Delta H_f}{(\Delta H_0)}\right)$$

ΔH_0 is the thermodynamic melting enthalpy of a 100% crystalline P(3HB) and this value is equal to 146 J/g. ΔH_f (J/g) is the melting enthalpy for the P(3HB) sample (the area under the melting peak) [21].

2.4.4.1.2 How the molar mass affects T_m, T_c and T_g

A high molecular weight polymer has a high viscosity at room temperature, a high melting point and a high crystallization temperature. A high molar mass polymer is more aligned in its solid state and a higher temperature is required to break the alignment and further induce the melt, compared to a low molar mass polymer. When a melted sample of a high molecular weight polymer is cooled, the crystallization may start earlier (at a higher temperature), than for a low molecular weight polymer. This because it is easier for long polymer chains to align and start the crystallization. Both T_m and T_c increases with increased molar mass [20].

If the amorphous part in a semi-crystalline sample is increased, the crystalline part becomes smaller and the crystallinity is decreased.

A high molecular weight polymer has a lot fewer chain ends compared to a low molecular weight polymer. Chain ends moves quite freely and if the number of chain ends is increased in a sample by reducing the molecular weight, this will lead to a lower T_m because less energy is required to energize chain motion and thereby melting.

The glass transition temperature decreases as the molar mass of a sample is lowered. Lower molecular weight means more free chain ends and these chain ends require more free volume to be able to move, than segments in the middle of the chain. When a polymer sample is heated, the chain ends can rotate and move more than the rest of the chain and this leads to a lower T_g.

A flexible chain has a lower T_g than a stiffer one. The explanation for this is that the energy required to make the flexible chain move is lower than for the stiff one. So, by increasing the rigidity of a chain, the T_g is raised. Another parameter that tends to increase T_g and the crystallinity for a sample is strong intermolecular forces [22].

3 Experimental Part

3.1 Materials

The P(3HB) biopolymer powder (ENMAT Y3000P) was purchased from Tianan Biologic Material Co. Ltd., Ningbo, China, and the P(3,4HB) biopolymer powder (SoGreen 00A-1) was purchased from Tianjin Green Bioscience, China. The fraction of 4HB units in the copolymer was 3 mol% [10].

Hydrochloric acid (37 wt%) and acetic acid (100 wt%) was available at CAS. Citric acid (100 wt%) and formic acid (95 wt%) was purchased from Sigma Aldrich [10].

3.2 Method

3.2.1 Washing, Filtration and Drying

The two biopolymers, P(3HB) and P(3,4HB), were washed with the same procedure, described below.

3.2.1.1 Washing

A mixing volume of 450 ml deionized water was poured into a beaker. The beaker was placed on a stir plate with a magnetic stirrer in it, see Figure 17. The right amount of acid was added to the beaker to get the desired concentration. 10 g of biopolymer powder was then added and the washing began, as Figure 18 show. The washing time was different for different experimental parts.

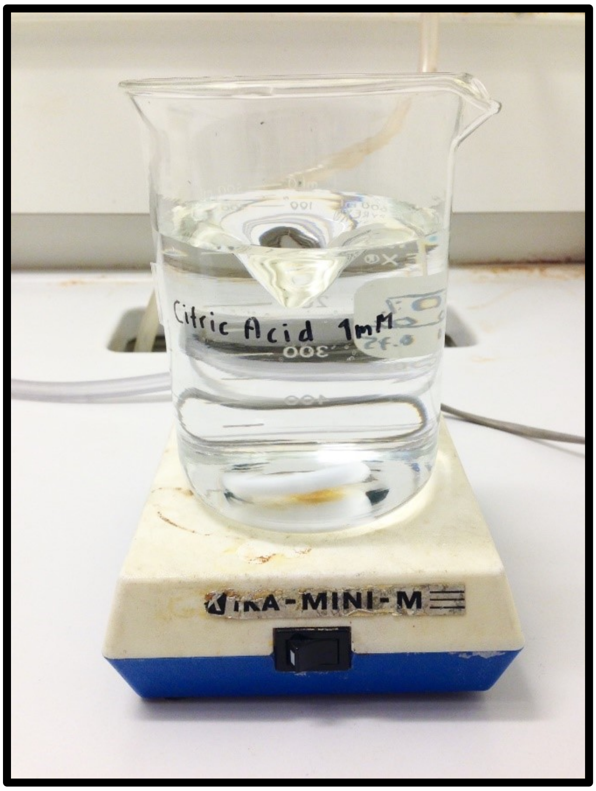


Figure 17. A beaker with acid solution placed on a magnetic stirrer to create a homogeneous mix of the deionized water, the acid and the biopolymer powder.

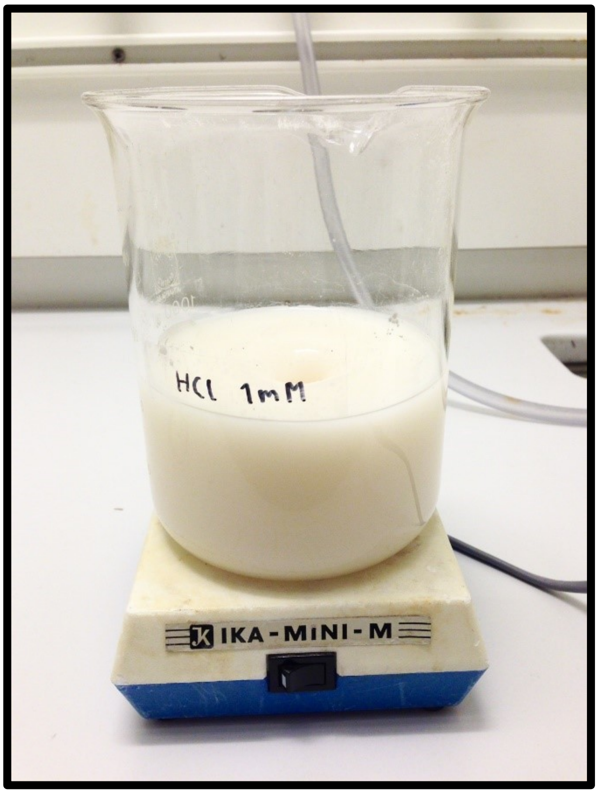


Figure 18. A beaker with a homogeneous mix of the acid solution and the added biopolymer powder.

3.2.1.2 Filtration

After the powder was washed with acid for a certain period of time, the powder solution was filtered through a glass filter using vacuum, and then washed three times with deionized water to make sure all acid was removed. See the filtration set-up in Figure 19.

Figure 19. Shows how the biopolymer powder was separated from the acid solution with the help of vacuum filtration.

3.2.1.3 Drying

The powder was then placed in a small glass container, sealed with a lid with tiny holes in it (Figure 27) and placed in a vacuum oven at 50°C for 24 h, see Figure 20. Half way through the experimental period the vacuum oven broke down. The problem was solved by drying the samples in a desiccator under vacuum at room temperature for 48 h, as Figure 21 show.

Figure 20. A vacuum oven where the filtered samples were placed at 50 °C for 24 hours to dry before any measurement could be made.

Figure 21. Desiccator with vacuum. Was used when the vacuum oven broke down. Since the temperature was room temperature, the samples were placed there for 48 hours.

3.2.2 Measurement Techniques

3.2.2.1 TGA

A TA Instruments TGA Q500 was used to study the degradation temperature of the samples.

A sample with a mass between three and six mg was analyzed. The temperature was raised with 10℃ per minute up to a temperature of 350℃, and the results were showed in the computer as the weight percent of the sample as a function of the temperature. Figure 22 shows how the sample was loaded into the instrument.

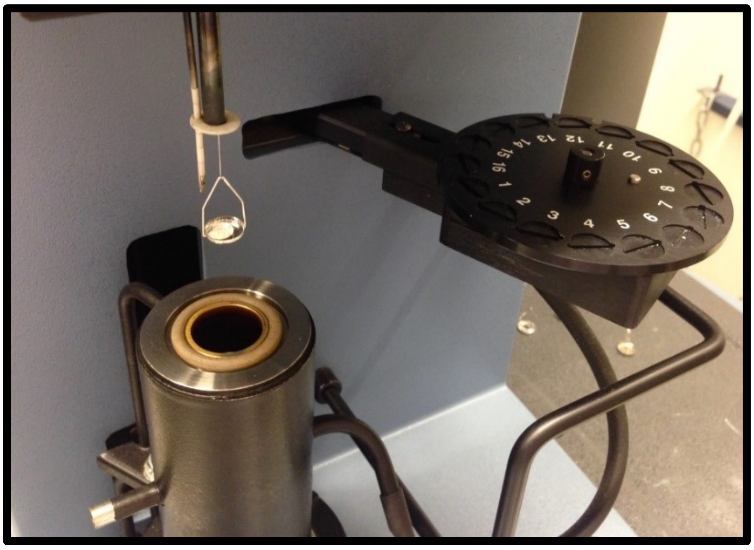


Figure 22. The TGA instrument. A sample being lowered into the oven for measurement is showed. The weight percent as a function of the temperature is shown in the computer.

3.2.2.2 Rheometer

A TA Instruments Advanced Rheometer AR 2000 ETC was used to study the modulus of the samples.

3.2.2.2.1 Hot press

Before using the rheometer, small tablets of 0.6 g powder were created using a cold and a hot press. The cold press, with a pressure of two bar, was used to create a powder tablet, see Figure 23. This tablet was then placed in the hot press that created a plastic tablet as in Figure 24. The pressure in the hot press was increased up to 10 bar during two minutes. The weight of the plastic tablet was used to calculate the thickness of it. This parameter was required in the rheometer settings.

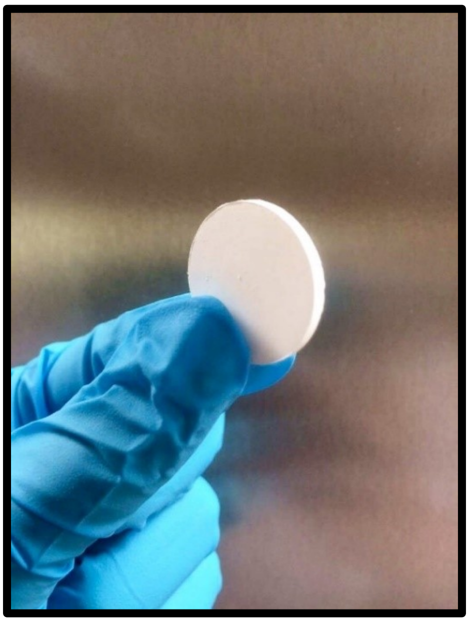


Figure 23. Powder tablet before prepared in the cold press.

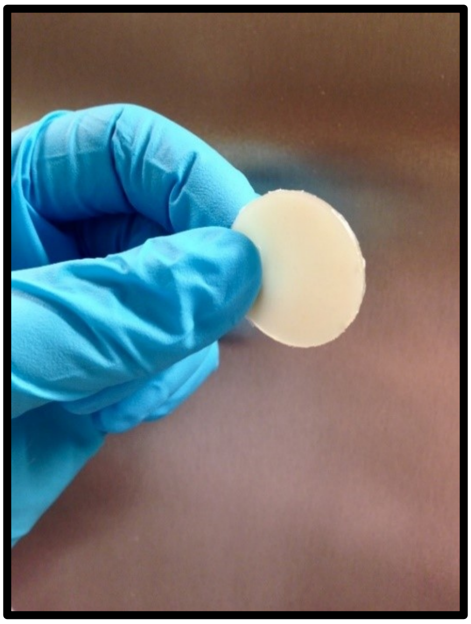


Figure 24. Plastic tablet prepared in the hot press. Was then placed in the rheometer.

3.2.2.2.2 Rheometer

The plastic tablet was placed in the rheometer as fast as possible to prevent a too large temperature drop. The parameter settings are shown in Table 2. The difference in temperature is due to the difference in melting temperature for the biopolymers.

Table 2. Parameter settings for the rheometer; temperature, time, frequency, and strain.

	Temperature (℃)	Time (min)	Frequency (Hz)	Strain (%)
P(3HB)	180	30	1	2
P(3,4HB)	170	30	1	2

Figure 25 shows a melted sample after being processed in the rheometer.

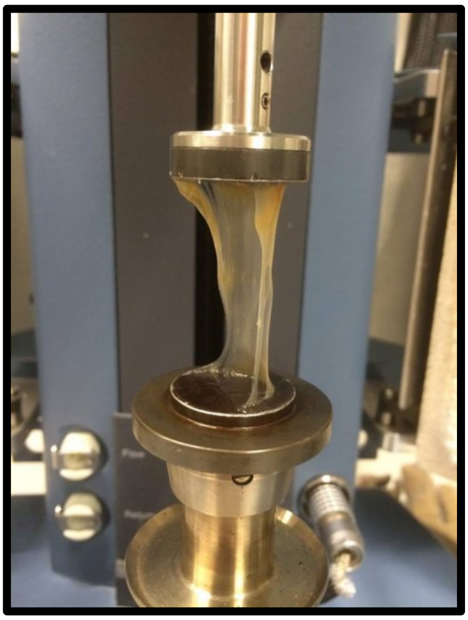


Figure 25. Melted plastic tablet after being processed in the rheometer.

3.2.2.3 DSC

A DSC Q 2000 was used to study the different phase transitions of the material during both cooling and heating.

The pan was filled with between 1 and 10 grams of biopolymer powder a lid was placed on the top. The sample was then placed in the DSC beside the reference (see Figure 26) and the analysis began. To erase the thermo-dynamic history, all the samples were heated and melted before the relevant measurement started. This was done by raising the temperature to 185℃. After the first heating, the sample was cooled to -70 °C followed by a second heating up to 200℃. Both the cooling rate and heating rate was 10℃/min, and this was done under nitrogen. During cooling, the crystallization temperature was shown and during heating, the melting temperature.

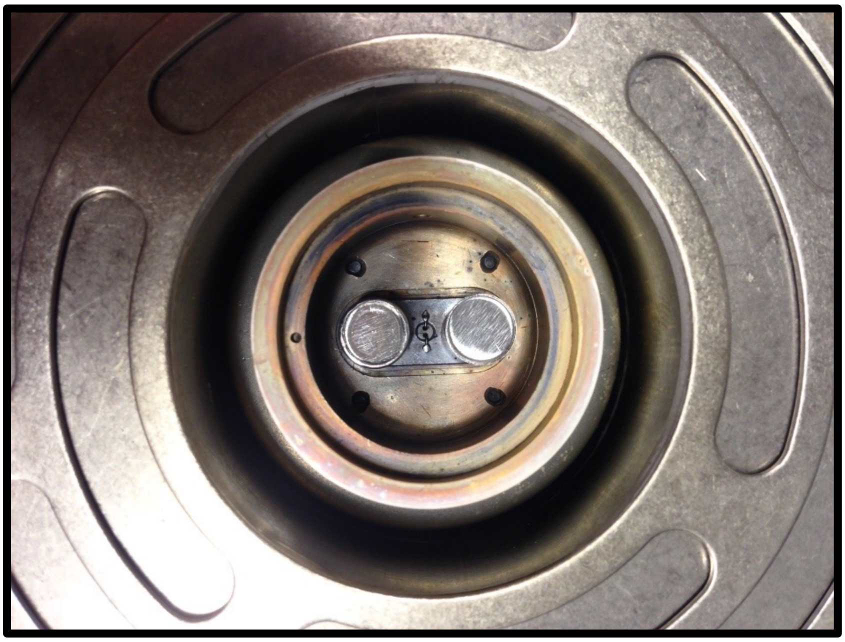


Figure 26. The sample (on the left) and the reference (on the right) placed in the DSC.

3.2.2.4 SEC

A SEC with three Shodex columns (KF-805, KF-804 and KF-802.5) connected to a Viskotek refractometer/viscometer 250 was used to study the molecular weight of the biopolymer material after rheology measurements. A calibration curve using polystyrene with four known molecular weights (M_w=650 000 g/mole, Water Associates; 96 000 g/mole, Polymer Laboratories; 30 000 g/mole, Polyscience Inc.; and 3180 g/mole, Agilent Technologies) was constructed [8].

To be able to perform SEC, the samples had to be dissolved in chloroform. A piece of around 30 mg polymer was placed in a vessel containing around 6 ml chloroform. This gives a concentration of 5 mg polymer/ml chloroform which was desired in this case. The exact weights and amounts of chloroform can be seen in Appendix 1. A syringe was used to load the SEC with a volume of 60 µl filtered solution.

A powder sample is shown in Figure 27 and a processed powder sample is shown in Figure 28. Figure 29 shows a processed powder sample dissolved in chloroform.

Figure 27. Powder sample after it has been acid treated, filtered and dried.

Figure 28. Processed powder sample in the rheometer.

Figure 29. Processed powder sample dissolved in chloroform.

3.3 Eight Experimental Parts

3.3.1 Experimental Overview

The experimental part was executed in eight steps. After executing one part, an evaluation was made to establish how to continue with the next part.

To be able to get a clearer view over the experimental part, a summary of all the washes and analyzes has been made for each biopolymer. In Figure 30, one can easily see which acid wash and further analyze that was made for the different P(3HB) samples. Figure 31 shows the experimental overview for the P(3,4HB) samples.

In the blue boxes, some sort of washing is described, and the green boxes state the analyzing method. The grey box is the as-received biopolymer powder.

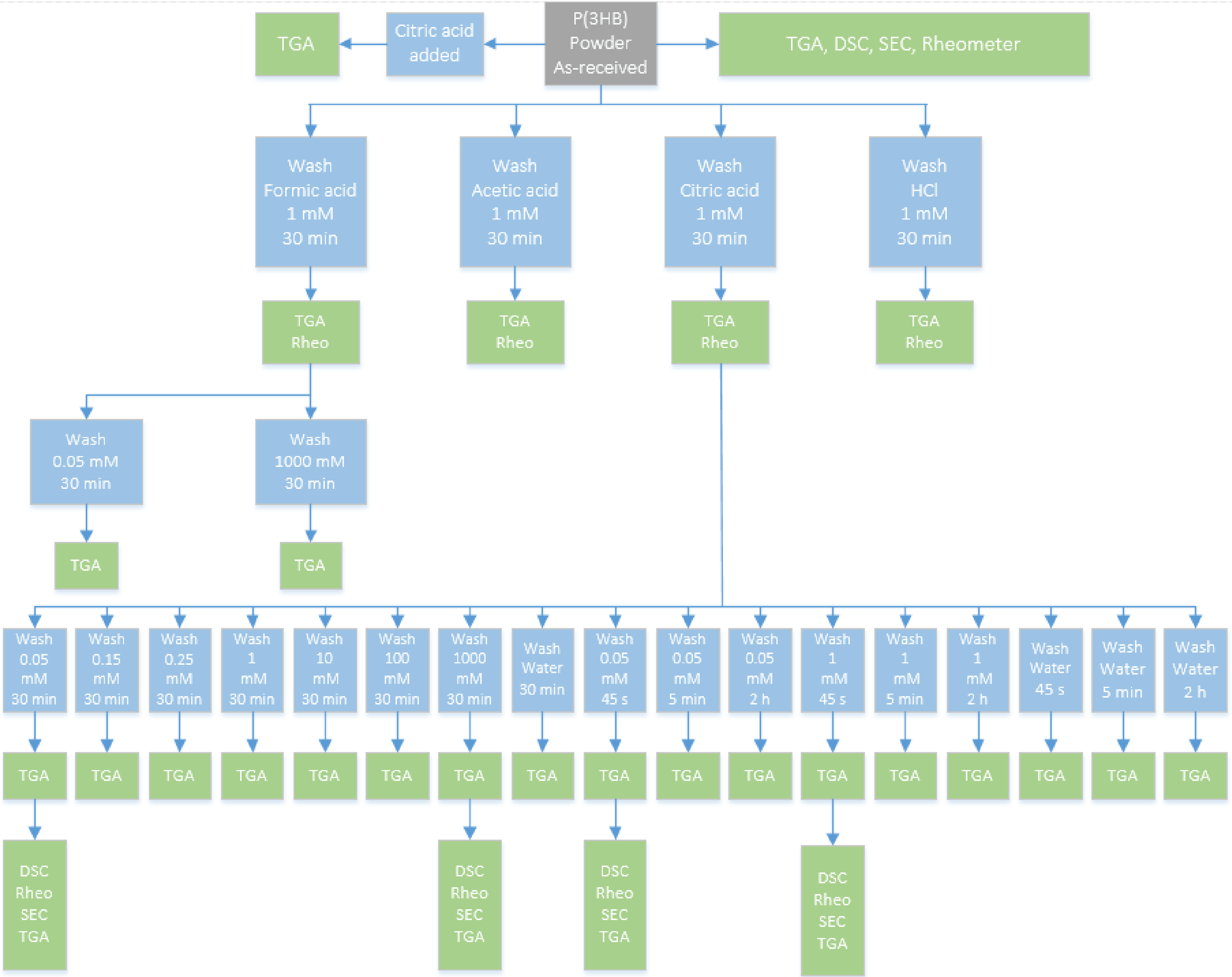


Figure 30. An experimental overview for the P(3HB) samples. The grey box showing the as-received sample, the blue boxes showing the washed samples, and the green boxes showing the analyzed samples.

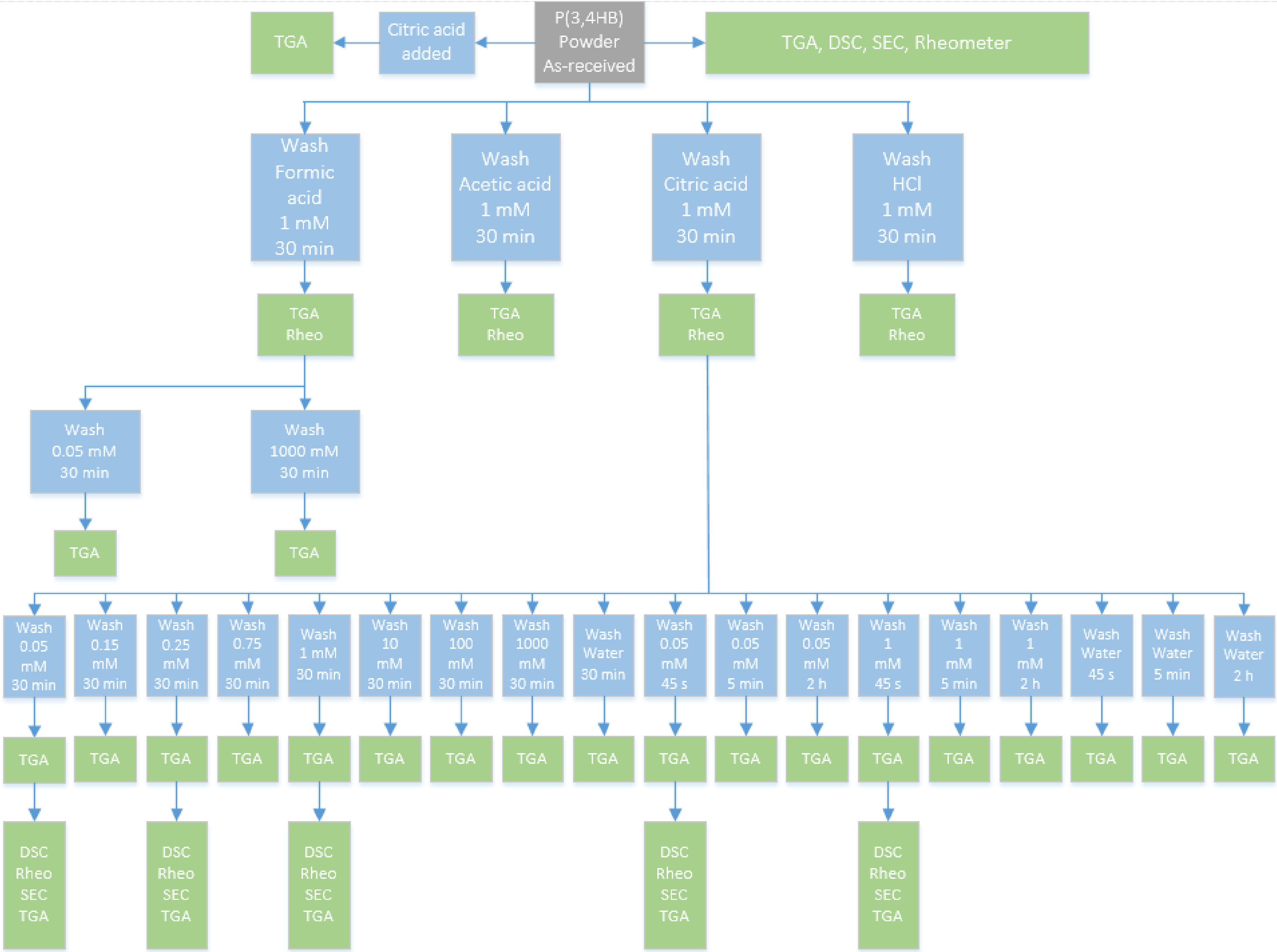


Figure 31. An experimental overview for the P(3,4HB) samples. The grey box showing the as-received sample, the blue boxes showing the washed samples, and the green boxes showing the analyzed samples.

3.3.2 Part 1 – Four Different Acids

According to earlier studies, a concentration of 1 mM was a suitable choice for the acid solutions [10]. In the first part, the two different biopolymers were washed with four different acids. Only one acid concentration and one washing time were studied. After washing, filtration and drying, the biopolymers were analyzed with TGA and rheometer to establish which acid that gave the most stable biopolymer.

The acids, washing times, concentrations, and amounts of acid powder/solution to give the right concentration are shown in Table 3. The equations and calculations used can be seen in Appendix 2.

Table 3. The acids, washing times, concentrations, and amounts of acid powder to give an acid solution of 1 mM.

Acid	Amount	Acid concentration (mM)	Washing time (min)
Citric acid	0.095 g	1	30
Hydrochloric acid	37.0 µl	1	30
Acetic acid	25.7 µl	1	30
Formic acid	894 µl	1	30

3.3.3 Part 2 – Citric Acid, Different Concentration

Based on the results obtained in part 1, citric acid and formic acid were chosen for further investigation for both biopolymers. Citric acid was the first acid to be studied and the experiments were performed using eight different concentrations. This was done to see how the concentration of the acid affected the biopolymer. The concentrations for formic acid was then chosen based on the result from the TGA of the citric acid, and further analyzed in part 5.

The samples were washed, filtered, and dried as described in the method. They were then analyzed with TGA and DSC.

The concentrations, amounts of citric acid, washing times, amounts of biopolymer powder and solution volume used for citric acid for both P(3HB) and P(3,4HB) can be seen in Table 4. Calculations for the amounts of citric acid can be seen in Appendix 3.

Table 4. Concentrations, amounts of citric acid, washing times, amounts of biopolymer powder and solution volumes used for both P(3HB) and P(3,4HB) experiments.

Concentration (mM)	Amount of citric acid (g)	Washing time (min)	Amount of biopolymer powder (g)	Solution volume (ml)
As-received	0	30	10	450
0.05	0.005	30	10	450
0.15	0.015	30	10	450
0.25	0.024	30	10	450
0.75	0.071	30	10	450
1	0.095	30	10	450
10	0.945	30	10	450
100	9.45	30	10	450
1000	94.5	30	10	450

3.3.4 Part 3 – Citric Acid, Washing Times

In this part, different washing times were investigated for two different citric acid concentrations to see the effect of time. This was made for both the biopolymer powders, and the samples were washed, filtrated, and dried as described in the method. They were then analyzed with TGA and DSC.

Table 5 shows the washing times, citric acid concentrations, amounts of biopolymer powder and solution volume.

Table 5. Washing times, concentration, amount of biopolymer powder and solution volume for both P(3HB) and P(3,4HB) experiments.

Washing times	Concentration (mM)	Amount of biopolymer powder (g)	Solution volume (ml)
45 s	C_1=0.05 C_2=1.00	10	450
5 min	C_1=0.05 C_2=1.00	10	450
30 min	C_1=0.05 C_2=1.00	10	450
2 h	C_1=0.05 C_2=1.00	10	450

3.3.5 Part 4 – Comparing Unprocessed and Processed Samples

Interesting results would be to see how some of the biopolymers from part 2 and 3 was affected when subjected to heat from the rheometer. Therefore, in this part, comparison between unprocessed and processed samples was made.

The samples for this part had already been prepared in part 2 and 3. After measurements with TGA and DSC had been made of the unprocessed powders, the samples were processed in the rheometer and then analyzed again with TGA, DSC and SEC.

The samples used from part 2 and 3 can be seen in Table 6 for P(3HB) and Table 7 for P(3,4HB).

Table 6. The citric acid concentrations of the five samples along with the washing times chosen for P(3HB).

	Concentration citric acid (mM)	Washing time
Sample 1	0	0
Sample 2	0.05	30 min
Sample 3	1000	30 min
Sample 4	0.05	45 s
Sample 5	1.00	45 s

Table 7. The citric acid concentrations of the six samples, along with the washing times chosen for P(3,4HB).

	Concentration citric acid (mM)	Washing time
Sample 1	0	0
Sample 2	0.05	30 min
Sample 3	0.25	30 min
Sample 4	1.00	30 min
Sample 5	0.05	45 s
Sample 6	1.00	45 s

3.3.6 Part 5 – Formic Acid, Different Concentrations

A fewer number of concentrations of formic acid was chosen for further investigation, based on the result from the TGA on citric acid. Only samples washed with concentrations that gave deviating results for citric acid were analyzed with formic acid.

The amount of liquid formic acid to add had to be calculated in two steps. This because the pipette used had a minimum volume of 20 µl, so a diluted solution had to be made first. Otherwise the preparations of the samples were made according to washing, filtration and drying in the method. These washed samples were only analyzed with TGA.

The formic acid concentrations, amounts of formic acid, washing times, amounts of biopolymer powder and solution volume used are shown in Table 8.

Table 8. The concentrations, amounts of formic acid, washing times, amounts of biopolymer powder and solution volume used for both P(3HB) and P(3,4HB) experiments.

Concentration (mM)	Amount of formic acid (µl)	Washing time (min)	Amount of biopolymer powder (g)	Solution volume (ml)
As-received	0	30	10	450
0.05 mM	45	30	10	450
1 mM	894	30	10	450
1000 mM	17900	30	10	450

3.3.7 Part 6 – As-received Powder with Citric Acid

To see if there was a need to wash away the citric acid added to the biopolymer powders, an experiment was made were a small amount of citric acid was mixed into the powder together with a small amount of water. The amount of water was tested out to get the right texture. The samples were then dried in vacuum for 48 hours without washing or filtration. To see the result of the acid present in the biopolymer powder, TGA was used.

Table 9 shows the amount of powder, citric acid, and water, and washing time used for both the biopolymers.

Table 9. The amount of powder, citric acid, and water and washing time for both P(3HB) and P(3,4HB) experiments.

Biopolymer	Amount of powder (g)	Amount of citric acid (g)	Amount of water (ml)	Washing time (min)
P(3HB)	2	0.02	4	0
P(3,4HB)	2	0.02	1.4	0

3.3.8 Part 7 – Water Wash

A wash with deionized water was executed to see the effect of the degradation temperature without any acid present. The two samples were prepared in the same way as described in the method chapter, apart from adding any acid. The biopolymer powders were then analyzed with TGA.

This part was executed in two steps because the samples in step 1 gave very interesting results that required further investigation with three more washing times.

3.3.8.1 Step 1

Table 10 shows the amounts of powder, citric acid and water, and washing time used for both the biopolymers.

Table 10. The amounts of powder, citric acid and water, along with the washing time for both P(3HB) and P(3,4HB) experiments.

Biopolymer	Amount of powder (g)	Amount of citric acid (g)	Amount of water (ml)	Washing time (min)
P(3HB)	10	0	450	30
P(3,4HB)	10	0	450	30

3.3.8.2 Step 2

Table 11 shows the amounts of powder, citric acid and water, and washing times used for both the biopolymers.

Table 11. The amounts of powder, citric acid and water, along with the washing times for both P(3HB) and P(3,4HB) experiments.

Biopolymer	Amount of powder (g)	Amount of citric acid (g)	Amount of water (ml)	Washing times
P(3HB)	10	0	450	45 s, 5 min, 2 h
P(3,4HB)	10	0	450	45 s, 5 min, 2 h

3.3.9 Part 8 – Error Sources

When the SEC results from part 4 was analyzed, it seemed like there might be an error since the results were very contradicting. An investigation of the possible error sources was therefore made as the end of the experimental parts. The molecular weight calculation process in the computer was repeated to see if that was a contributing factor to the unclear results. Also, an investigation was made where a new piece of the processed sample (positioned in a different place on the plastic sample) was selected, dissolved, and analyzed in the SEC.

In the DSC, all the samples were heated to a temperature of 185°C, and this may have affected the P(3,4HB) samples improperly since this temperature is too high above its melting temperature of around 170°C.

The DSC and TGA results with processed powder may also depend on which piece of the sample that was selected.

4 Results and Discussion

4.1 Important Results Obtained from Each Method

Before the results are presented, a small summation of the most important results from each analyzing method has been made.

4.1.1 TGA

The curves from the TGA showed how the sample weight was changed with increased temperature. The degradation temperature was obtained at the highest slope of the curve.

4.1.2 Rheometer

The storage modulus, |G'|, is a measurement of the resistance against deformations, and shows the stiffness of the material. The longer the biopolymer is exposed to a specific temperature, the more the polymer chains breaks down into smaller chains and the viscosity decreases which lowers the strength. A decrease in the slope for the storage modulus curve during time indicates that the biopolymer has become more stable.

Delta is the difference between input and output. Delta has a value between 0 and 90, where 0 equals an elastic, solid material and 90 equals a newtonic, liquid material.

4.1.3 SEC

The curves received from this measurement was calibrated with a polystyrene standard with four known molecular weight (600 000, 96 000, 30 000 and 3180 g/mol), to give the unknown molecular weight of the sample. This was done to see how the molecular weight of the polymer chains have been affected after processed in the rheometer.

4.1.4 DSC

Important parameters obtained from the DSC were glass transition temperature, melt temperature, crystallization temperature, melt/crystallization enthalpies, and crystallinity. In some cases, more than one melt- and crystallization temperature for the copolymer P(3,4HB) was showed. Two melting peaks correspond to a melting-recrystallization-remelting process [10]. Two crystallization temperatures (one during cooling and one during heating) might depend on that the cooling rate was too high, the chains were not given enough time to align and crystallize. Instead, the crystallization occurs during heating.

4.2 Part 1 – Four Different Acids

The results from TGA and rheology analysis of P(3HB) and P(3,4HB) are shown in Figure 32-36 and Table 12-13 below.

4.2.1 P(3HB)

4.2.1.1 TGA

The TGA results for P(3HB) is shown in Figure 32 and Table 12.

Figure 32 shows the weight percent as a function of temperature for the different samples.

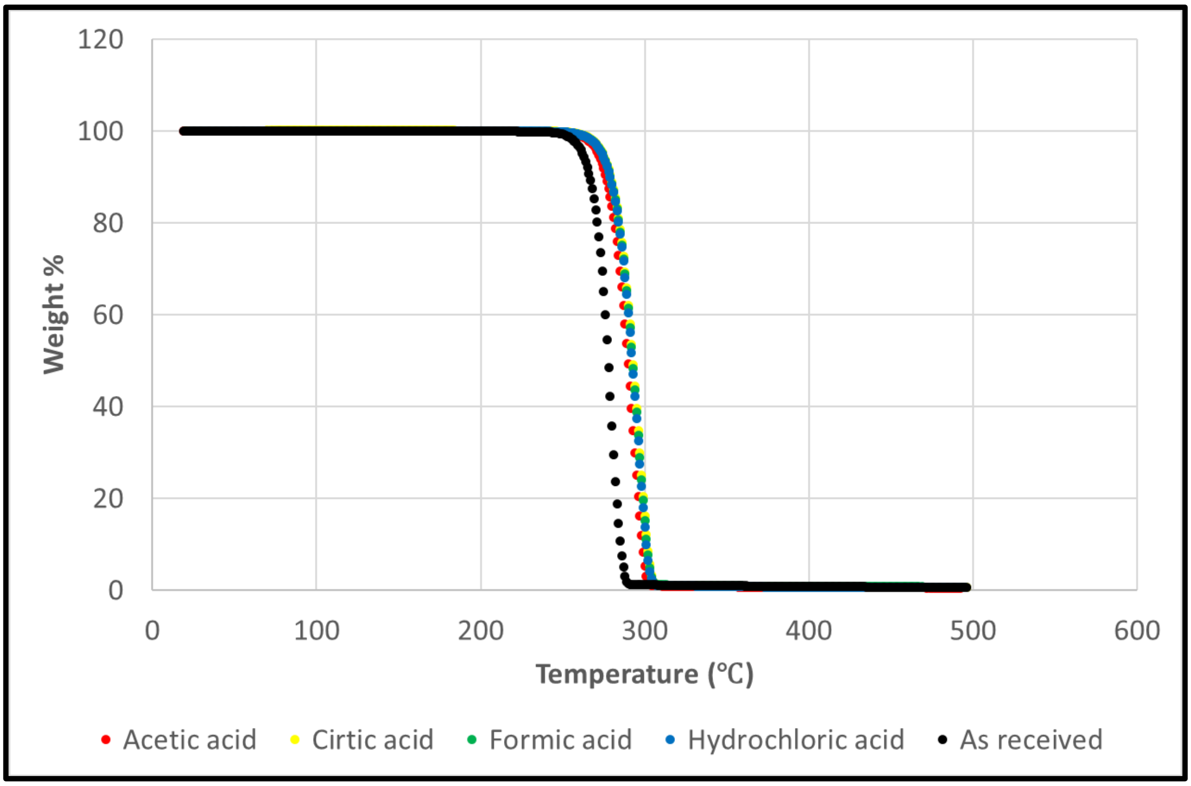


Figure 32. TGA curves for the P(3HB) powder washed with 1 mM acetic acid, citric acid, formic acid and hydrochloric acid, along with the as-received P(3HB) powder. The weight percent is shown as a function of temperature, and displays the degradation temperatures.

From Figure 32, the degradation temperatures for the different samples were obtained and can be seen in Table 12.

Table 12. The degradation temperatures of both the as-received biopolymer powder and the powders washed with four different acids; acetic acid, citric acid, formic acid and hydrochloric acid.

Acids used for washing	Degradation temperature (℃)
Acetic acid	295
Citric acid	296
Formic acid	295
Hydrochloric acid	295
As-received	279

As can be seen from both Table 12 and Figure 32, there was a little difference in the degradation temperatures between the different acids used for washing. Washing with citric acid gave the highest degradation temperature, but only 1℃ above the other acids.

A possible explanation for the fact that all the acids provided almost the same result was that the concentration used for the acids was enough to remove all the accessible impurities, and therefore, the results became very similar.

There was however a ~16℃ difference between the as-received powder and the acid washed powders which told us that the degradation temperature was increased when P(3HB) was washed with acid. The increased degradation temperature most likely depended on the fact that the impurities had been minimized from the biopolymer powder when washed with acid, which made the biopolymer more thermally stable.

4.2.1.2 Rheometer

Figure 33 shows the results from the rheology analysis, the modulus on the left y-axis and delta on the right one.

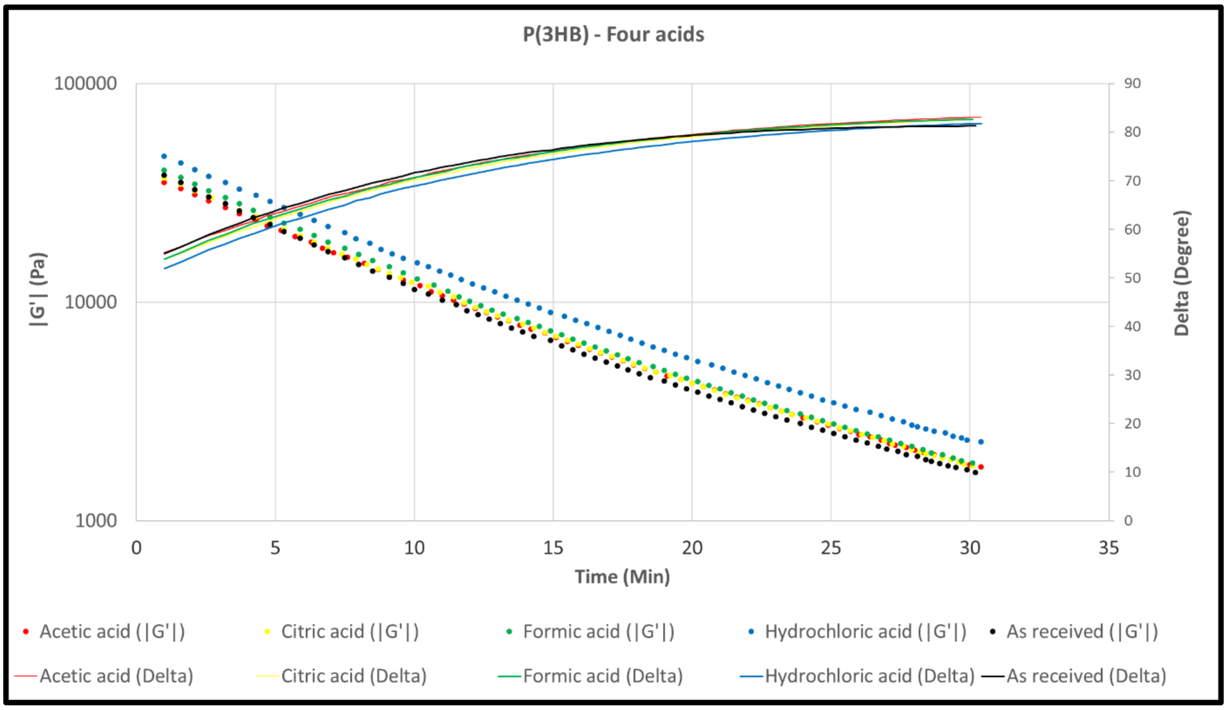


Figure 33. The five modulus- and delta curves from rheology measurements for the four acid washed P(3HB) powders, along with the as-received P(3HB). The modulus and the delta are shown as a function of time. The acids used for washing was acetic acid, citric acid, formic acid and hydrochloric acid.

As can be seen, all the P(3HB) samples, washing with different acids and as-received, resulted in curves with a similar appearance.

The powder washed with hydrochloric acid had a modulus located at a higher level in the diagram compared to the other samples. But the slope of the curve was the same as for the others, and this meant that there was no significant difference in the results between the acids. As Figure 33 show, the slope of the modulus curve of the as-received powder was a bit greater than for the other samples. This indicated that this material was more unstable and the stiffness changes with time.

Since the modulus curves showed a similar appearance it was expected to get a similar appearance between the delta curves as well, which was the case. The delta curves showed an increased delta value with time which meant that the material became more viscous and less elastic.

4.2.2 P(3,4HB)

4.2.2.1 TGA

The TGA results for P(3,4HB) is shown in Figure 34 and Table 13.

Figure 34 shows the weight percent as a function of temperature for the different samples.

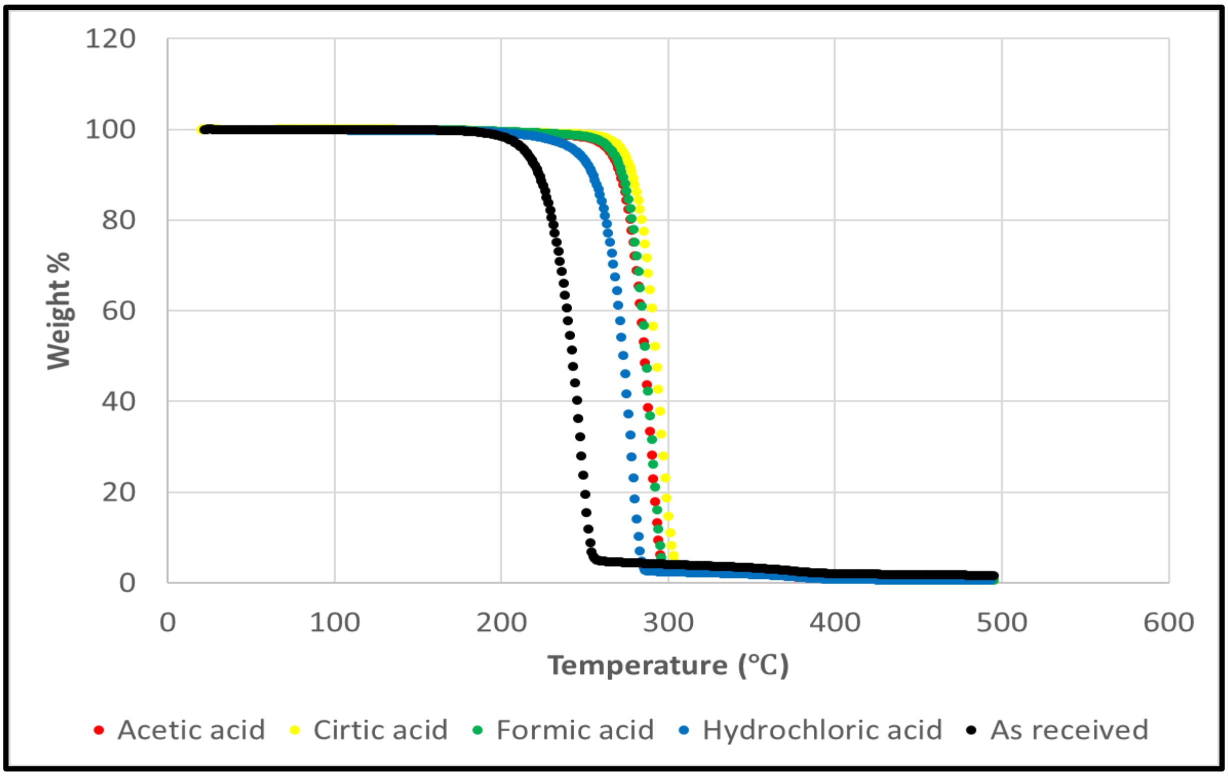


Figure 34. The TGA curves from four acid washed P(3,4HB) powders and one as-received P(3,4HB) powder. The weight percent is shown as a function of temperature, and displays the degradation temperatures. The acids used are citric acid, formic acid, acetic acid and hydrochloric acid.

From Figure 34, the degradation temperatures for the different samples were obtained and can be seen in Table 13.

Table 13. The received degradation temperatures from TGA measurements of both the as-received biopolymer powder, P(3,4HB) and the powders washed with four different acids; citric acid, formic acid, acetic acid and hydrochloric acid.

Acids used for washing	Degradation temperature (°C)
Acetic acid	289
Citric acid	295
Formic acid	289
Hydrochloric acid	277
As-received	247

Here, a difference in degradation temperature was obtained between the powders washed with different acids, which may have indicated that this material was more sensitive than P(3HB). The powder washed with citric acid gave the highest degradation temperature. This may have been a result of the chelating effect mentioned in the theory. The powders washed with acetic acid and formic acid gave a similar degradation temperature. This was probably because they had similar properties. The lowest degradation temperature, apart from the as-received, was given by the powder washed with hydrochloric acid. This result seemed a bit odd since hydrochloric acid was the strongest acid used and based on earlier studies, the degradation temperature was improved with around 50°C by washing with a 1 mM hydrochloric acid solution for 30 minutes [10]. In this case, the degradation temperature was only improved with 30°C. A possible explanation for this might have been that the strength of the acid solution was irrelevant in this case.

4.2.2.2 Rheometer

The P(3,4HB) results are shown in Figure 35.

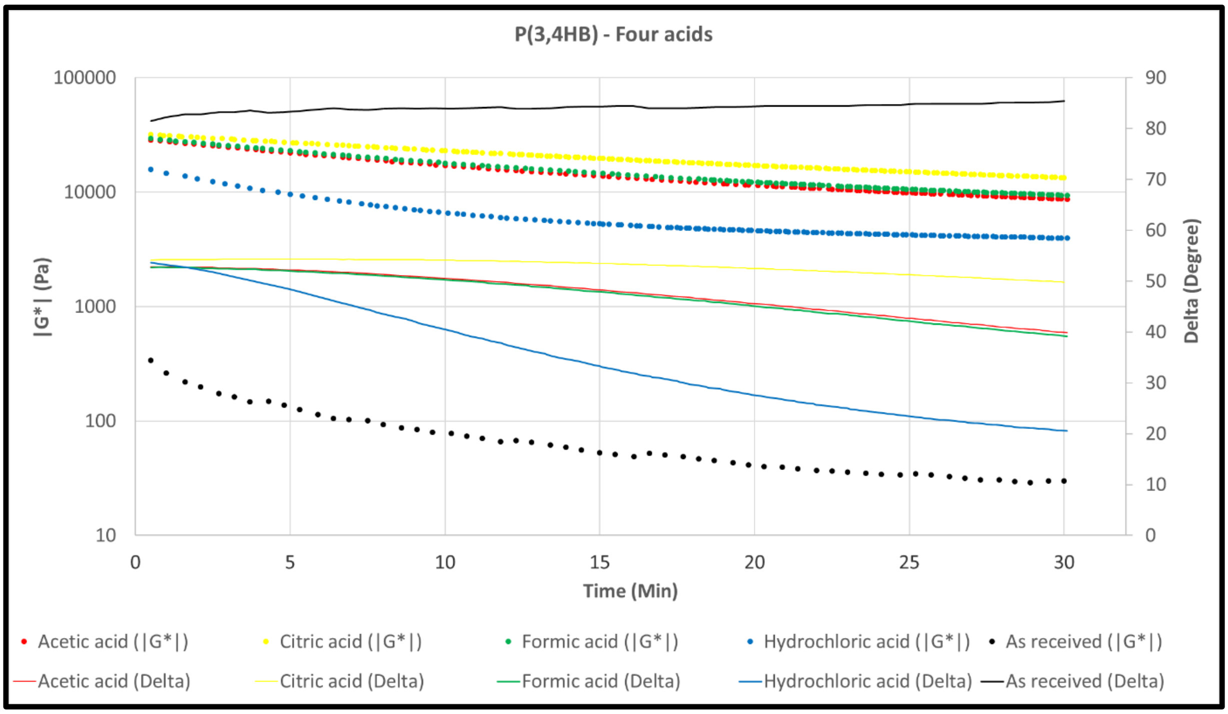


Figure 35. The five modulus- and delta curves from rheology measurements from four acid washed P(3,4HB) and one as received. The modulus and the delta are shown as a function of time. The acids used for washing was acetic acid, citric acid, formic acid and hydrochloric acid.

Here, a big difference was obtained between both the modulus and the delta curves. As can be seen in the diagram for the modulus curves, the as-received powder had a much lower modulus compared to the curves received from the powders washed with acid. One explanation why the as-received powder had a much lower modulus in the beginning of the analysis was because the material started to degrade in a higher extent than the other samples during the making of the tablet in the hot press. When the results from the acid washed powders were compared, hydrochloric acid gave a modulus curve with the highest slope while citric acid gave a curve with a lower slope. This indicated that washing with citric acid resulted in the most stable material, and washing with hydrochloric acid resulted in the most unstable material. A reduction in modulus could be related to polymer chain scission reactions. A reduction in molecular weight implies a reduction in melt viscosity and therefore the dynamic shear modulus. These curves corresponded well to the curves received from the TGA.

When looking in Figure 35 for the delta curves, the as-received powder gave a curve located in the top of the diagram, with an increased delta value with time. This indicated that the material became less viscous during time. The curves received from the powders washed with acid gave curves where the delta was decreased with time. Hydrochloric acid gave a curve located at the lowest position in the diagram, the value decreased more by time compared to the other acid washed powers. The decrease in the delta slopes may have depended on the fact that a network was formed during processing, which would result in a more elastic material. This however needed to be further investigated before established.

4.2.3 Comparing TGA and Rheometer Results

4.2.3.1 P(3HB)

The results from the TGA and rheometer analyze, when looking at which acid was the most effective, corresponded quite well to each other. One exception was that the as-received sample differed from the other samples in the TGA, but not in the rheometer analysis where it followed the other samples well. Also, the hydrochloric acid had a higher located modulus than the other curves in the rheometer analyze which corresponded to a higher molecular weight.

4.2.3.2 P(3,4HB)

The TGA and rheometer analysis gave the same results when looking at the stability of the samples. The as-received powder had the lowest stability followed by powder washed with hydrochloric acid, formic/acetic acid, and citric acid.

4.3 Part 2 – Citric Acid, Different Concentrations

The results from part 2 were analyzed with TGA, DSC and rheometer. TGA was examined and evaluated first. DSC and rheometer was only used on samples that showed interesting and different TGA results.

All the results are shown in Table 14-19, and in Figure 36-47.

4.3.1 P(3HB)

4.3.1.1 TGA

The curves from the TGA measuring are shown in Figure 36. The degradation temperature for the sample washed with different concentrations was obtained.

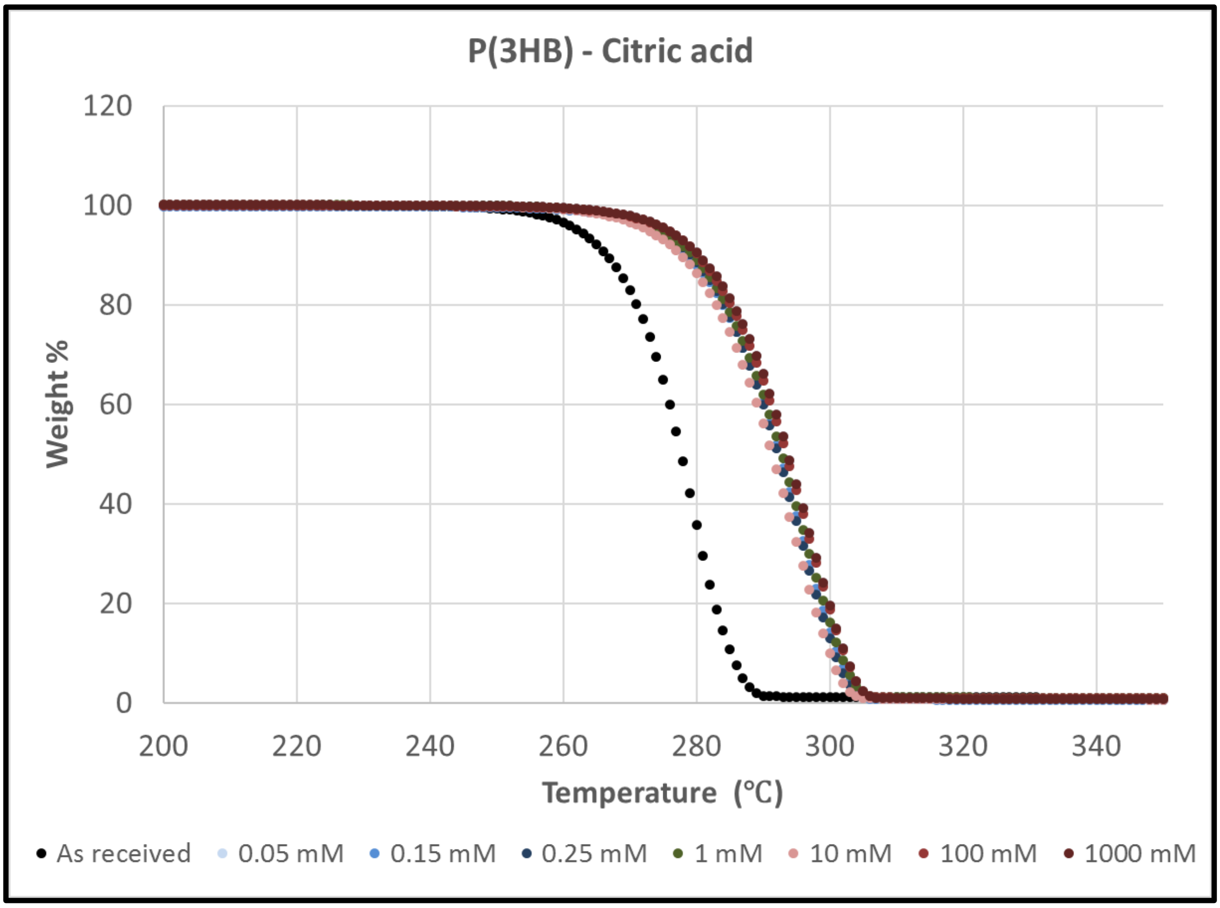


Figure 36. TGA results from P(3HB) powder washed with seven different citric acid concentrations, 0.05 mM, 0.15 mM, 0.25 mM, 1.00 mM, 10 mM, 100 mM and 1000 mM, together with as-received powder. The weight percent is shown as a function of temperature, and the degradation temperature is displayed.

Table 14 shows the seven different concentrations of citric acid and their corresponding degradation temperatures, together with the as-received sample.

Table 14. The degradation temperatures for the as received powder and for the powders washed with different citric acid concentrations; 0.05 mM, 0.15 mM, 0.25 mM, 1.00 mM, 10 mM, 100 mM and 1000 mM

Concentration (mM)	Degradation temperature (℃)
As-received	279
0.05	296
0.15	296
0.25	295
1	296
10	295
100	295
1000	295

In Figure 37 and 38, the data from Table 14 was plotted with the degradation temperature as a function of the concentration. To be able to see the behavior at low concentrations, the data has been divided into two plots due to the big difference in concentration.

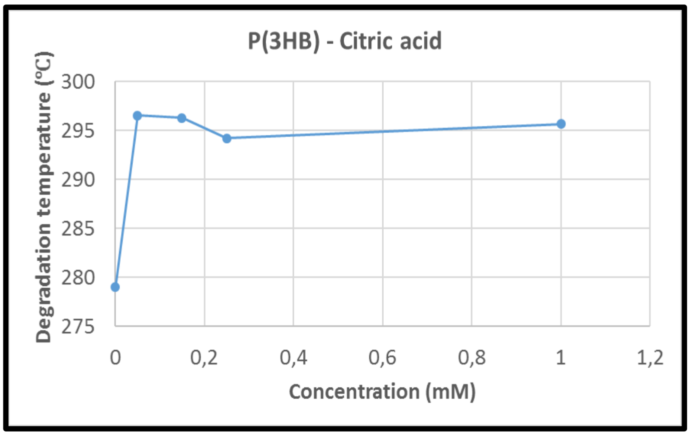


Figure 37. Citric acid concentrations with the range from 0 – 1 mM, showing the increase of the degradation temperature when more citric acid is added.

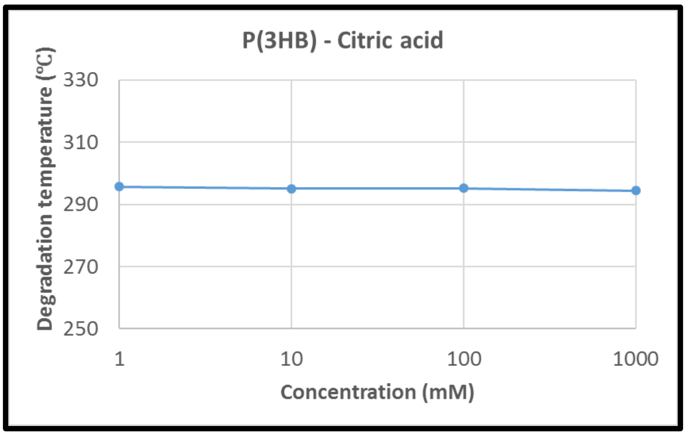


Figure 38. Citric acid concentrations with the range from 1 – 1000 mM, showing the effect of the degradation temperature when more citric acid is added.

In Figure 37, the degradation temperature for P(3HB) was highly increased when washed with 0.05 mM citric acid. A further increase in the citric acid concentration would not improve the degradation temperature.

When looking at Figure 38 where very high acid concentrations was used, the degradation temperature was not improved. The degradation temperature reached a maximum at around 295℃ with an acid concentration of 1 mM. The conclusion drawn from this was that when washing P(3HB) with an acid concentration of 0.05 mM citric acid, as much impurities as possible were removed and there was no point to raise the concentration more.

4.3.1.2 DSC

Measurements of both unprocessed powder and of powder processed in the rheometer were made.

In Table 15 and 16, the melt-, crystallization- and glass transition temperatures (T_m, T_c and T_g), the enthalpy of melting (ΔH_m), enthalpy if crystallization (ΔH_c), and the crystallinity of the sample (X_c) is shown. The calculation of the crystallinity is shown in Appendix 3.

Figure 39 and 40 are the curves from the DSC measurements, were the table-values was taken form.

4.3.1.2.1 Unprocessed Powder

Table 15 shows the parameters for the unprocessed powder; melting temperature, crystallization temperature, glass transition temperature, melt enthalpy, crystallization enthalpy, and crystallinity.

Table 15. Six different parameters received from the DCS for three unprocessed samples; as-received, 0.05 mM and 1000 mM.

	T_m (°C)	T_c (°C)	T_g (°C)	ΔH_m (J/g)	ΔH_c (J/g)	X_c (%)
As-received	176	123	3.85	94	88	64
0.05 mM	177	122	2.92	78	70	53
1000 mM	176	122	3.98	99	87	68

The values in Table 15 comes from Figure 39 that shows the heating and cooling cycles for the as-received powder, and two other samples washed with different citric acid concentrations, 0.05 and 1000 mM.

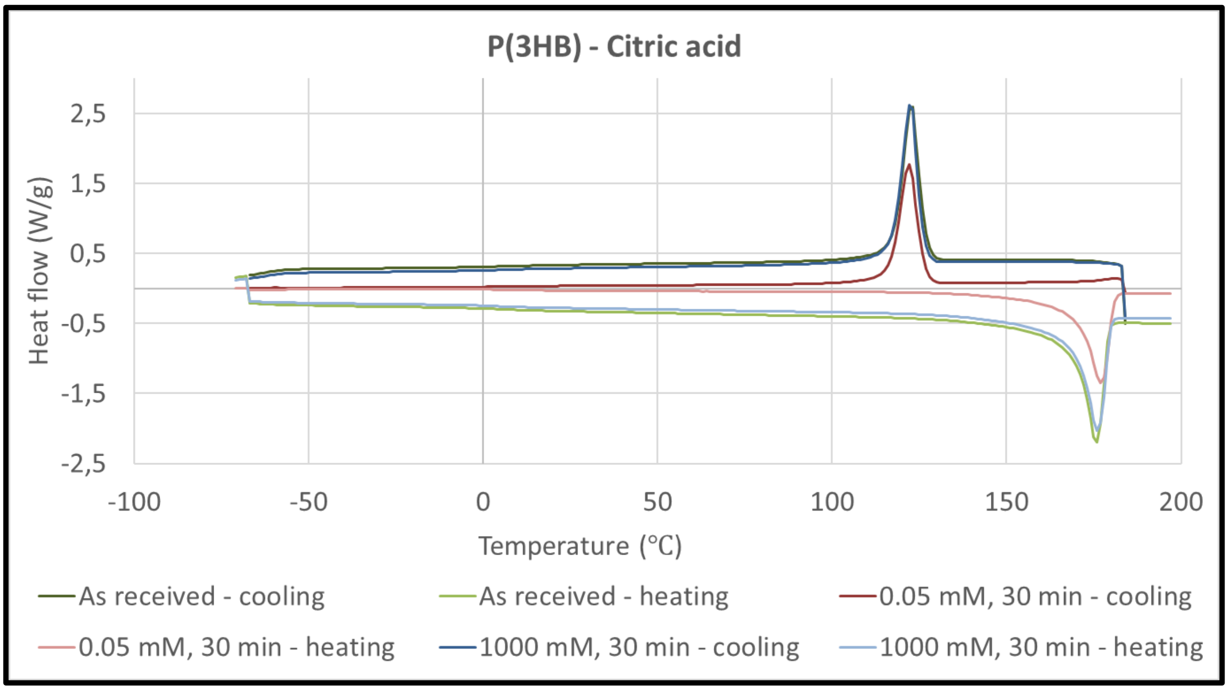


Figure 39. The DSC heating and cooling cycles for three different samples; as-received, 0.05 mM 30 min, and 1000 mM 30 min. The heat flow is shown as a function of the temperature.

As can be seen in Table 15 above, the melting- and crystallization temperatures for the three samples were as much as the same. However, the melting- and crystallization enthalpies differed. The 0.05 mM sample had the lowest enthalpies, while the as-received sample and the 1000 mM sample had almost the same.

4.3.1.2.2 Processed Powder

Table 16 shows the properties of P(3HB) after processed and analyzed in the rheometer; melting temperature, crystallization temperature, melt enthalpy, crystallization enthalpy, and crystallinity.

Table 16. Six different parameters received from the DCS for three processed samples; as-received, 0.05 mM and 1000 mM.

	T_m (℃)	T_c (℃)	T_g (℃)	ΔH_m (J/g)	ΔH_c (J/g)	X_c (%)
As-received	172	125	5.82	94	88	64
0.05 mM	175	124	6.39	104	99	71
1000 mM	175	125	4.83	95	88	65

The values in Table 16 were given by the curves in Figure 40.

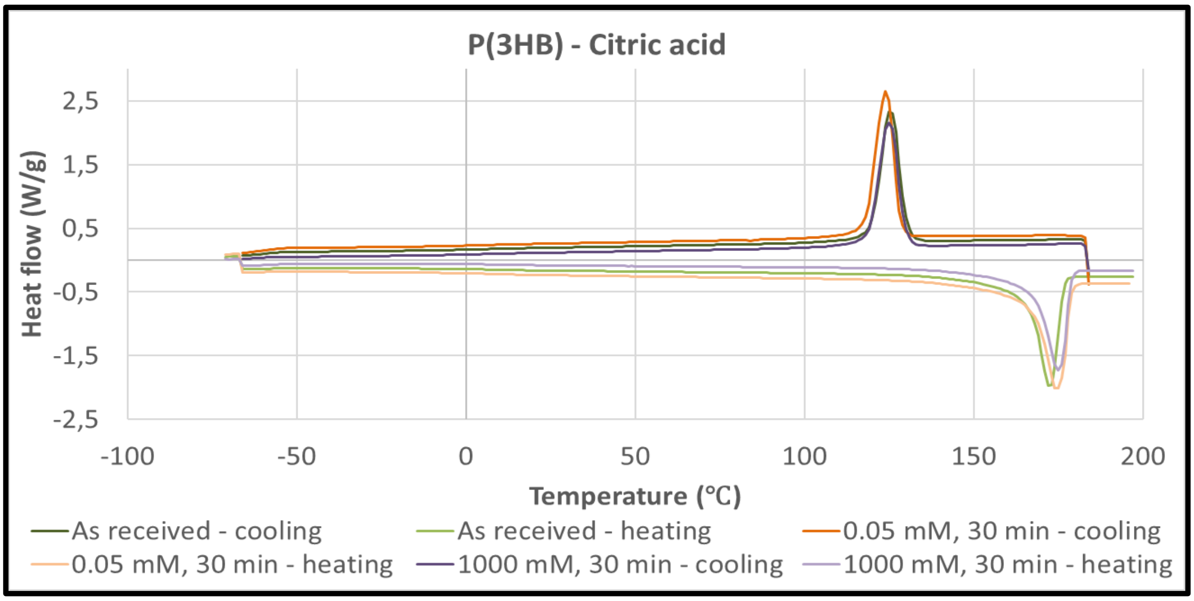


Figure 40. The DSC heating and cooling cycles for three different samples; as-received, 0.05 mM 30 min, and 1000 mM 30 min. The heat flow is shown as a function of the temperature.

The samples after processing also had very similar melting- and crystallization temperatures. But here, the enthalpies for the 0.05 mM sample was the highest and the enthalpies for the as-received sample and the 1000 mM sample lowest was very similar. Here, there was no offset that could explain the difference. Since the enthalpies for as received and 1000 mM samples was almost the same, it was odd that the 0.05 mM sample did not gave similar results.

4.3.1.3 Rheometer

The results from the two different citric acid concentrations along with the as-received powder were also analyzed with rheometer and these results can be seen in Figure 41.

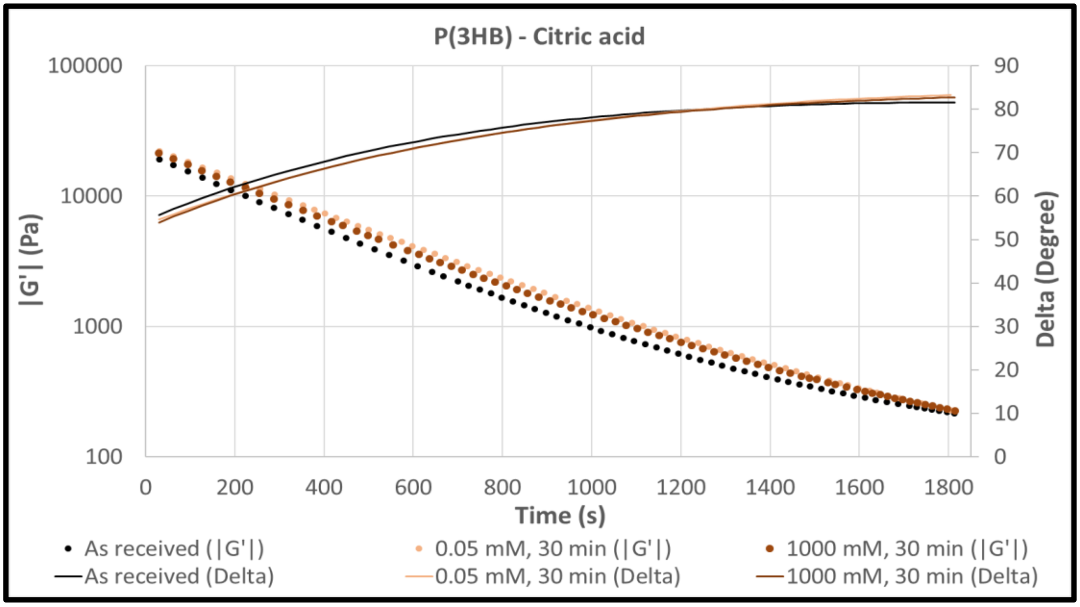


Figure 41. The three modulus- and delta curves from rheology measurements for the two acid washed P(3HB) powders, along with the as-received P(3HB). The modulus and the delta are shown as a function of time. The acid used for washing was citric acid.

The modulus curves for the samples were very alike. They started and finished at proximately the same point. The two washed samples had however a somewhat straighter curve which indicated that these biopolymers had become more stable. Based on the TGA results, the washed samples should have had a higher thermal stability.

4.3.2 P(3,4HB)

4.3.2.1 TGA

The curves from the TGA are shown in Figure 42. The degradation temperature for the samples washed with different concentrations was obtained.

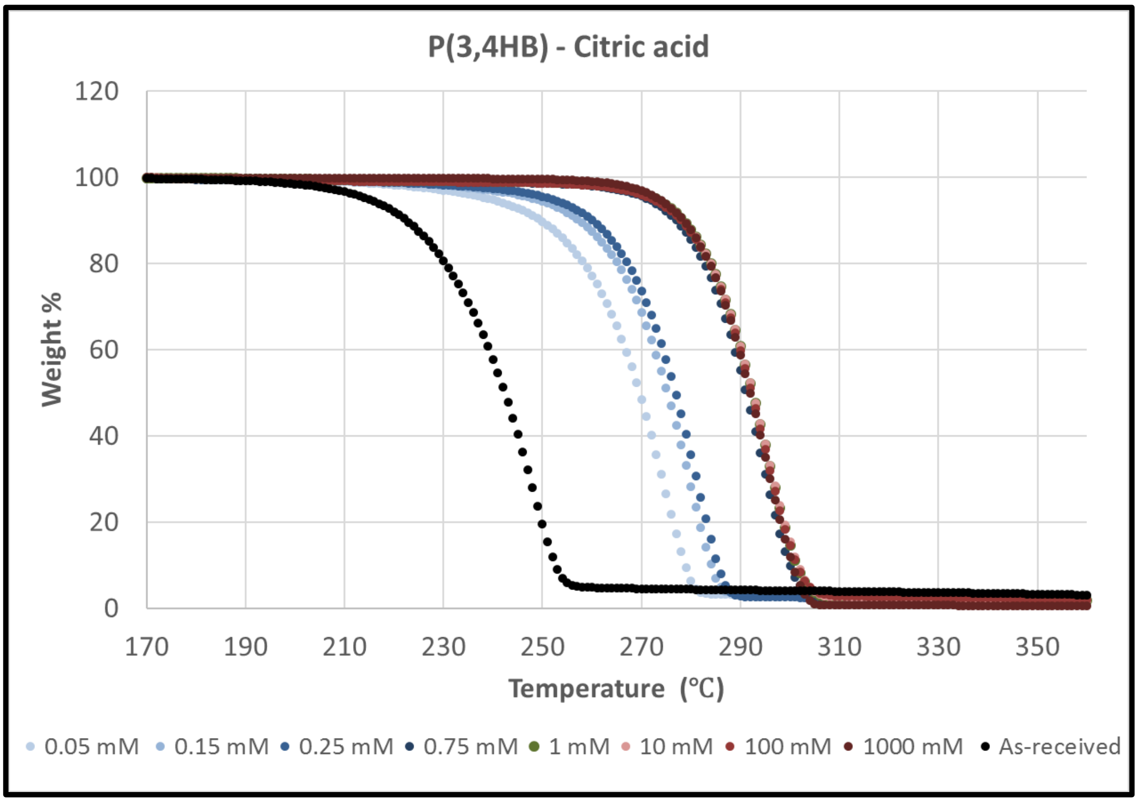


Figure 42. TGA traces of eight different citric acid concentrations, 0.05 mM, 0.15 mM, 0.25 mM, 1.00 mM, 10 mM, 100 mM and 1000 mM, together with as-received powder, are shown with their corresponding degradation temperatures. The weight percent is shown as a function of temperature.

In Table 17, the eight different citric acid concentrations, together with the as-received powder, are shown with their corresponding degradation temperatures.

Table 17. The degradation temperatures for the powders washed with eight different citric acid concentrations; 0.05 mM, 0.15 mM, 0.25 mM, 1.00 mM, 10 mM, 100 mM and 1000 mM, together with as-received powder.

Concentration (mM)	Degradation temperature (℃)
As-received	247
0.05	274
0.15	279
0.25	281
0.75	294
1	295
10	295
100	295
1000	295

Two plots were the degradation temperature was plotted against citric acid concentration can be seen in Figure 43-44.

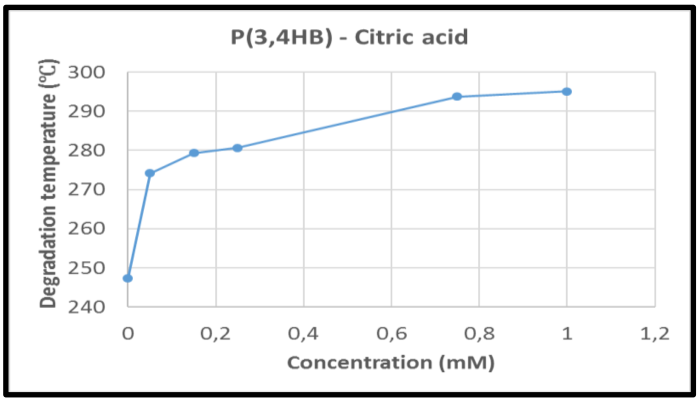


Figure 43. Variation of degradation temperature with citric acid concentrations with the range from 0 – 1 mM, showing the increase of the degradation temperature when more citric acid is added.

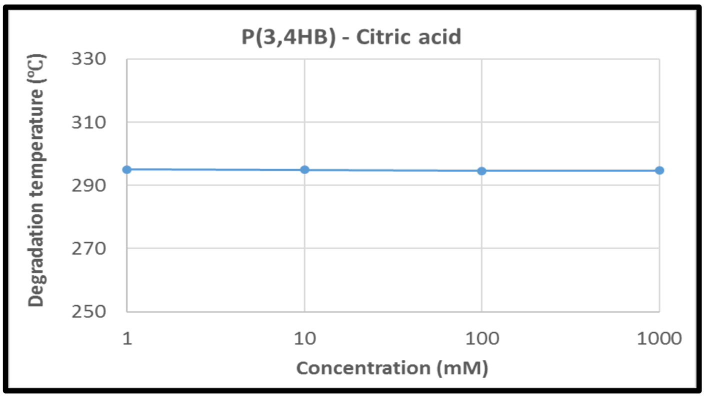


Figure 44. Variation of degradation temperature with citric acid concentrations with the range from 1 – 1000 mM, showing the increase of the degradation temperature when more citric acid is added.

Figure 43 shows that washing P(3,4HB) with different citric acid concentrations gave a successive increase in degradation temperature from 0.05 mM to 1 mM. A higher concentration than 0.05 mM for P(3HB) was needed to get the highest obtained degradation temperature for P(3,4HB). P(3,4HB) did not reach its maximum degradation temperature at 295℃ until it was washed with a solution of 1 mM citric acid. This might tell us that the impurities were stronger attached to the copolymer, or/and that it may contained a different kind of impurities that was harder to wash away. It may also be that the copolymer was quite pure to begin with.

4.3.2.2 DSC

Also for the copolymer, measurements of unprocessed powder and powder processed in the rheometer were made to see how the biopolymer properties changed after being subjected to heat.

For the copolymer, two melting temperatures (T_m) and mostly two crystallization temperatures (T_c) was obtained. The two melting temperatures corresponded to a melting-recrystallization-remelting process. T_c corresponds to the crystallization temperature received when the sample is crystallized during cooling. In this case, not all the sample was able to crystallize during the cooling, so the rest of the sample crystallizes when heated again (T_{cc}). Also, a glass transition temperature (T_g) was found. ΔH_m stands for the energy required to melt the sample, and ΔH_c is the energy required to crystallize the sample (ΔH_c during cooling and ΔH_{cc} during heating).

4.3.2.2.1 Unprocessed Powder

Table 18 shows different parameters received from the DSC.

Table 18. The different parameters received from the DCS for four unprocessed samples; as-received, 0.05 mM, 0.25 mM and 1.00 mM.

	$T_{m,1}$ (°C)	$T_{m,2}$ (°C)	T_c (°C)	T_{cc} (°C)	T_g (°C)	ΔH_m (J/g)	ΔH_c (J/g)	ΔH_{cc} (J/g)	X_c (%)
As-received	131	149	49	41	-1.9	69	12	27	47
0.05 mM	145	160	78	47	1.7	74	39	6.5	51
0.25 mM	144	159	58	44	1.7	74	38	5.6	51
1.00 mM	145	159	77	37	1.6	73	39	7.5	50

Figure 45 shows the heating and cooling cycles for the as-received sample, and for three more samples washed with different citric acid concentrations.

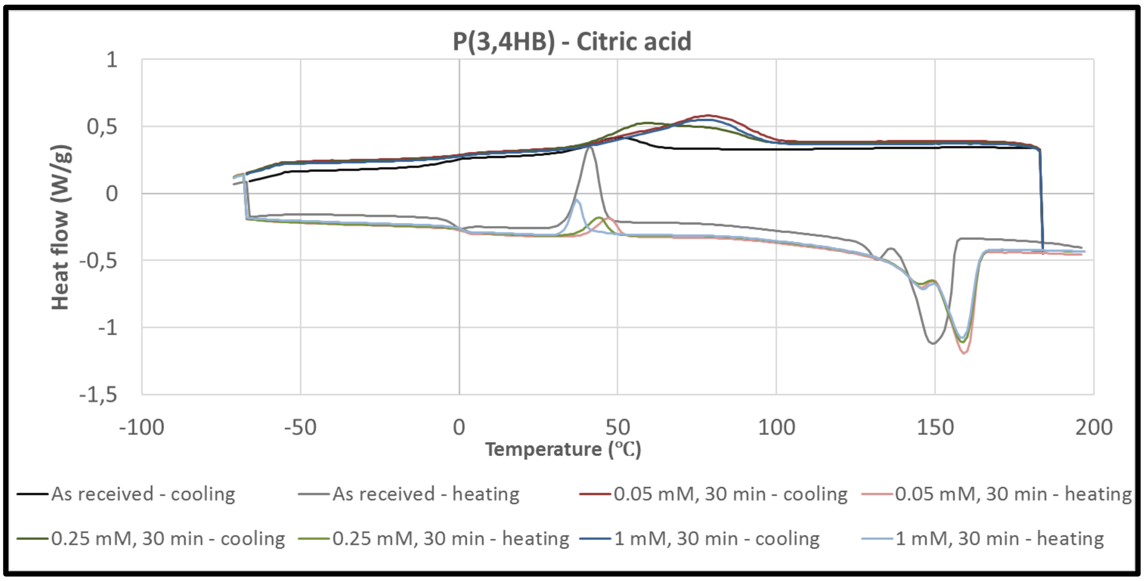


Figure 45. The DSC heating and cooling curves from samples washed with different concentration of citric acid; as-received, 0.05 mM 30 min, 0.25 mM 30 min, and 1.00 mM 30 min. The heat flow is shown as a function of temperature.

Table 18 shows that the melting temperatures were higher for the samples washed with citric acid (~14℃). The crystallization temperatures differed a bit and did not follow a specific pattern. The glass transition temperature was lowest for the as-received sample. The melting enthalpies for all the samples were almost the same. The crystallization enthalpies were higher for the acid washed samples compared to the as received sample, and the cold crystallization enthalpies was lower for the acid washed samples compared to the as received.

The cold crystallization showed in some of the DSC curves may have depended on the inability of the crystallisable chains to completely crystalize during the cooling of the sample.
A possible reason for this was that the cooling rate was too high and the polymer chains were not given enough time to align and rearrange in a crystalline structure. The polymer crystalizes during heating instead, when more time was given for the polymer to arrange in the most stable confirmation.
Another reason for this might be that the viscosity for the melt was very high, so the polymer chains moved very slow and had therefore not enough time to organize themselves.

4.3.2.2.2 Processed Powder

Table 19 shows the results of the biopolymer powder after being processed in the rheometer.

Table 19. The different parameters received from the DCS for four processed samples; as-received, 0.05 mM, 0.25 mM and 1.00 mM.

	$T_{m,1}$ (℃)	$T_{m,2}$ (℃)	T_c (℃)	T_{cc} (℃)	T_g (℃)	ΔH_m (J/g)	ΔH_c (J/g)	ΔH_{cc} (J/g)	X_c (%)
As-received	107	125	-	57	-15	48	-	45	33
0.05 mM	144	156	93	-	1.2	74	65	-	51
0.25 mM	153	153	107	-	2.0	71	65	-	49
1.00 mM	155	165	110	-	3.0	73	65	-	50

The information in Table 19 comes from the heating- and cooling cycles in Figure 46.

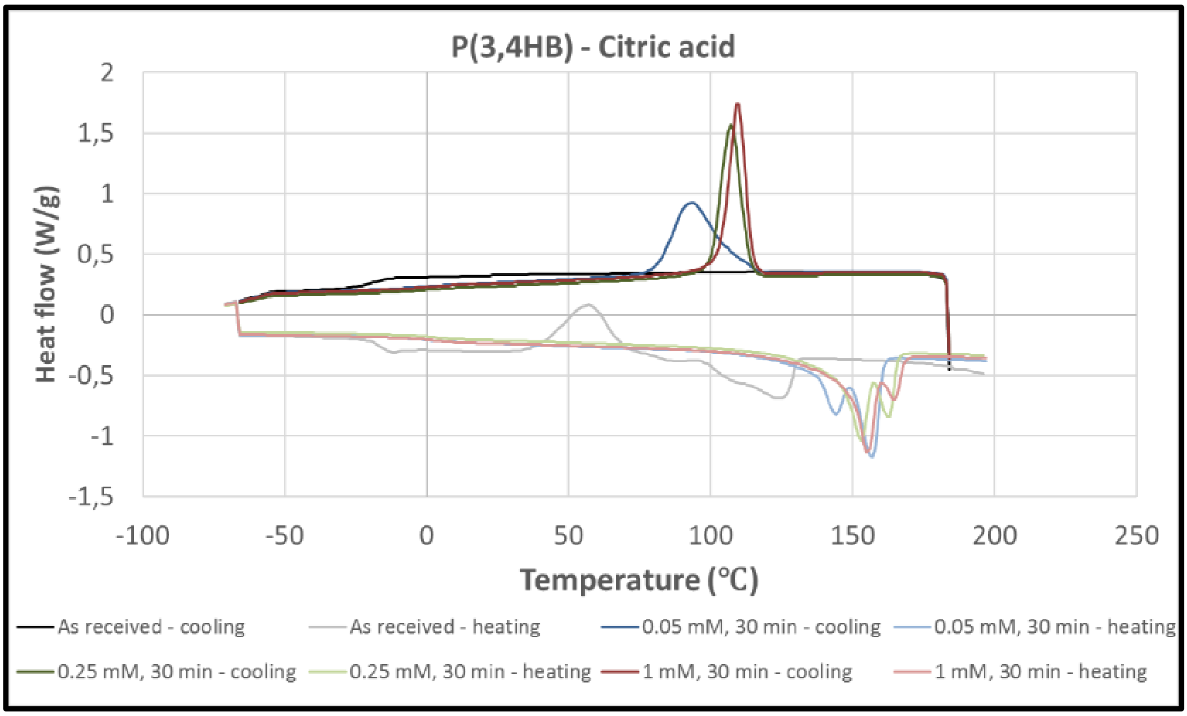


Figure 46. The DSC heating and cooling curves for three different samples as-received, 0.05 mM 30 min, 0.25 mM 30 min, and 1.00 mM 30 min. The heat flow is shown as a function of temperature.

Both $T_{m,1}$ and $T_{m,2}$ were higher for the acid washed samples. No crystallization temperature existed for the as-received sample, unlike the acid washed samples, where the crystallization temperatures were increased with increasing acid concentration. No cold crystallization temperature existed for the acid washed samples. The glass transition temperature was lowest for the as-received sample. The melting enthalpies were almost the same for the acid washed samples, and higher than for the as-received sample.

4.3.2.3 Rheometer

The results obtained from the rheometer are shown in Figure 47.

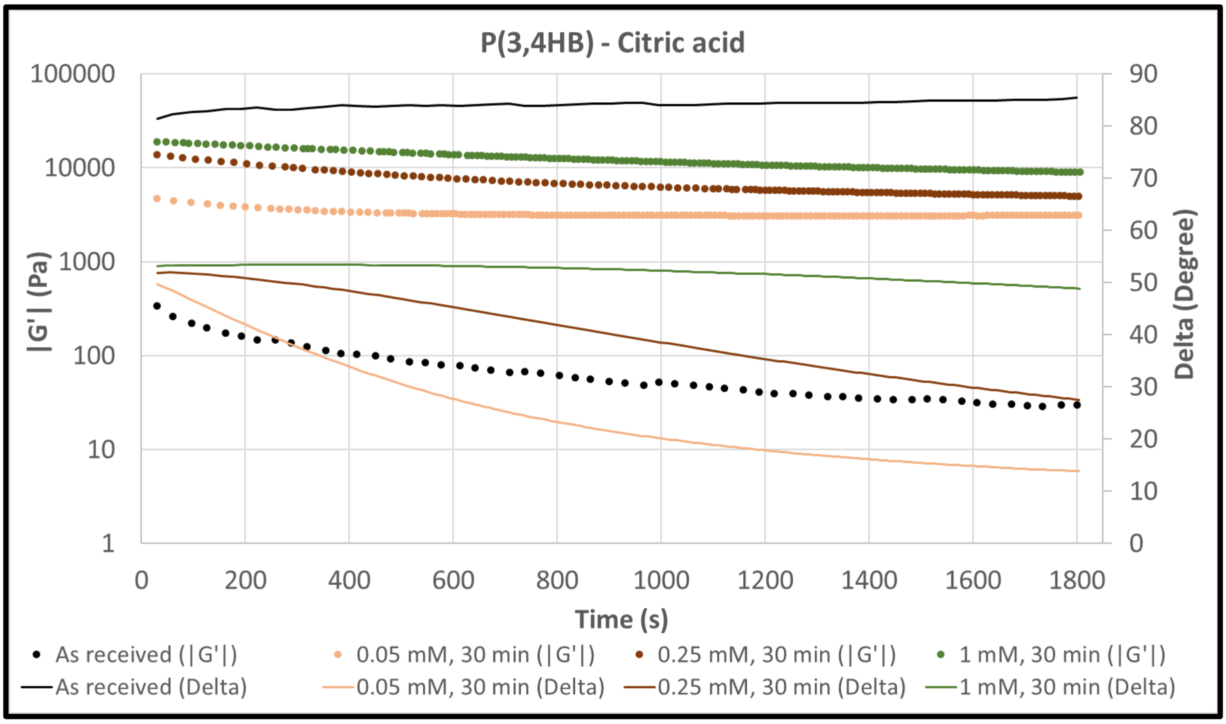


Figure 47. The four modulus- and delta curves from rheology measurements for the two acid washed P(3HB) powders, along with the as-received P(3HB). The modulus and the delta are shown as a function of time. The acid used for washing was citric acid.

As can be seen in the figure above, the modulus curve for the as-received sample had a lower location than the modulus curves for the washed samples. This tells us that the thermal stability was increased when the samples had been washed with acid. Although here, the 0.05 mM, 30 min sample had the lowest slope of the modulus curve which means that this sample was the most stable. Although it was hard to know the change of the storage modulus before the rheometer analyze was performed since the degradation started in the hot press.

For the delta curves, the washed samples had much lower located curves than the as-received sample. This should mean that the viscosity and the elasticity was increased for the washed samples. An increased viscosity indicates a higher molecular weight and strength, which was desirable.

4.4 Part 3 – Citric Acid, Washing Time

The results from part 3 were obtained with TGA, DSC and rheometer analysis. Only the most interesting result was analyzed further in the DSC and rheometer.

4.4.1 P(3HB)

4.4.1.1 TGA

The degradation temperatures received from the four different washing times in two different citric acid concentrations, 0.05 mM, and 1.00 mM are shown in Table 20.

Table 20. The degradation temperatures received from different washing times (45 s, 5 min, 30 min and 2 h) for two different citric acid concentrations (0.05 mM and 1.00 mM).

	45 s	5 min	30 min	2 h
0.05 mM	295℃	295℃	297℃	294℃
1.00 mM	294℃	296℃	296℃	295℃

As can be seen, a longer washing time for both the concentrations had no effect on the degradation temperature. The maximum value was reached already at 0.05 mM and 45 seconds. This means that there was no need for either high citric acid concentration or long washing times to get a good result.

4.4.1.2 DSC

45 seconds was enough time to achieve more thermally stable biopolymers, so only these samples were investigated further.

In the DSC, measurements of unprocessed powder and powder processed in the rheometer were made to see how the biopolymer properties changed after being subjected to heat and movement for a longer time.

In Table 21 and 22, the melt-, crystallization- and glass transition temperatures (T_m,T_c and T_g), the enthalpy of melting (ΔH_m) and enthalpy of crystallization (ΔH_c), and the crystallinity of the sample (X_c) is shown. The calculation of the crystallinity is shown in Appendix 3.

Figure 48 and 49 are the resulting curves from the DSC measurements, were the values in Table 21 and 22 are based on.

4.4.1.2.1 Unprocessed Powder

Table 21 shows the parameters for the unprocessed powder.

Table 21. The different parameters received form the DCS for three unprocessed samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s.

	T_m (°C)	T_c (°C)	T_g (°C)	ΔH_m (J/g)	ΔH_c (J/g)	X_c (%)
As-received	176	123	3.85	94	88	64
0.05 mM, 45 s	176	123	5.32	88	89	60
1.00 mM, 45 s	176	123	2.41	104	91	72

Figure 48 below show the heating and cooling curves for the as-received sample, and concentrations 0.05 and 1000 mM washed for 45 seconds.

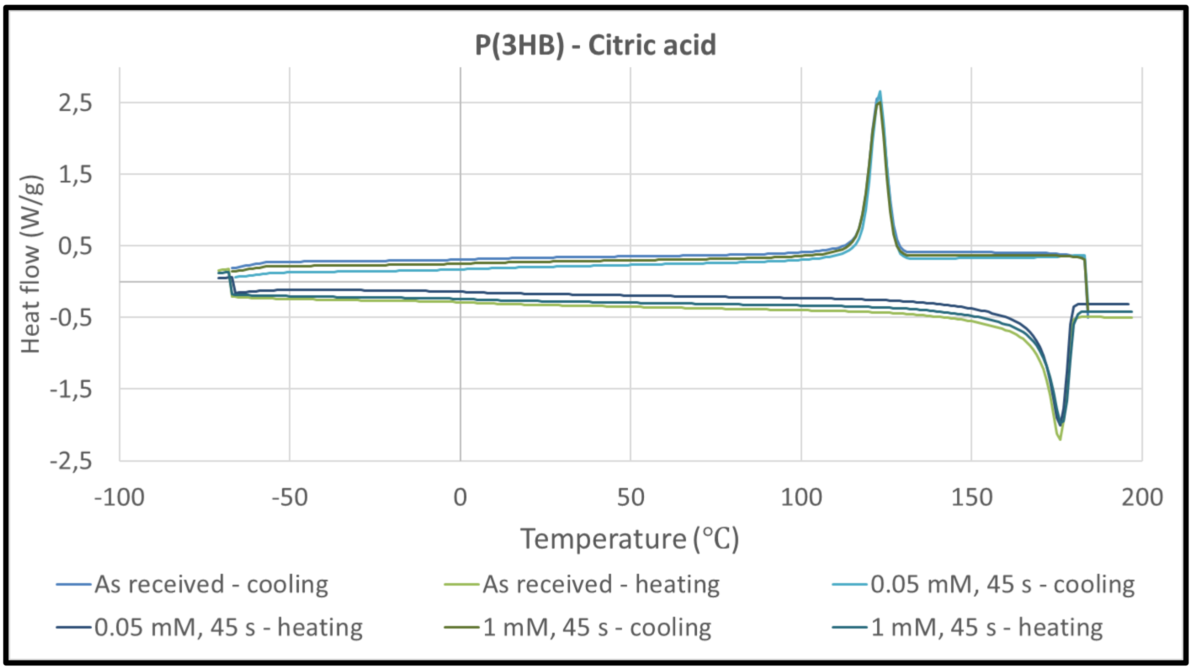


Figure 48. The heating and cooling curves for three different samples; as-received, 0.05 mM 45 s and 1.00 mM 45 s. The heat flow is shown as a function of temperature.

The melting- and crystallization temperatures were exactly alike for all the three samples. The other parameters were also close to each other.

4.4.1.2.2 Processed Powder

Table 22 shows the properties of the biopolymer after processing in the rheometer.

Table 22. Three different parameters received from the DCS for three processed samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s.

	T_m (°C)	T_c (°C)	T_g (°C)	ΔH_m (J/g)	ΔH_c (J/g)	X_c (%)
As-received	173	125	5.82	94	88	64
0.05 mM, 45 s	173	115	3.28	97	90	66
1.00 mM, 45 s	175	120	7.11	96	92	66

The values in Table 22 are based on the curves from Figure 49.

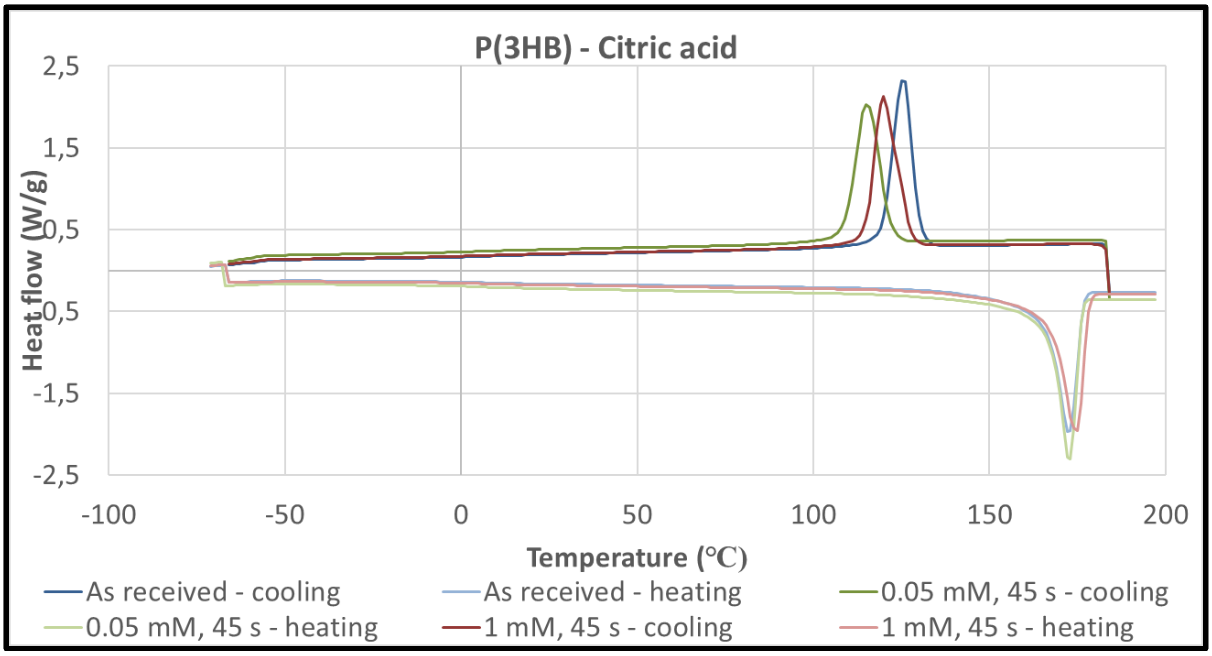


Figure 49. The DSC heating and cooling cycles for three different samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s. The heat flow is shown as a function of temperature.

After the samples have been processed in the rheometer, the properties differ. The melting temperatures were somewhat alike, but the crystallization temperatures drifted apart. The as-received sample had the highest crystallization temperature and the 0.05 mM, 45 s the lowest. The temperature results in Table 22 were very contradicting because the highest melting temperature did not correspond to the highest crystallization temperature. Also, this contradicted the theory that states that a sample with a low molecular weight should have had a low melting- and crystallization temperature.

4.4.1.3 Rheometer

The rheometer results for the 45 second samples are shown in Figure 50 below.

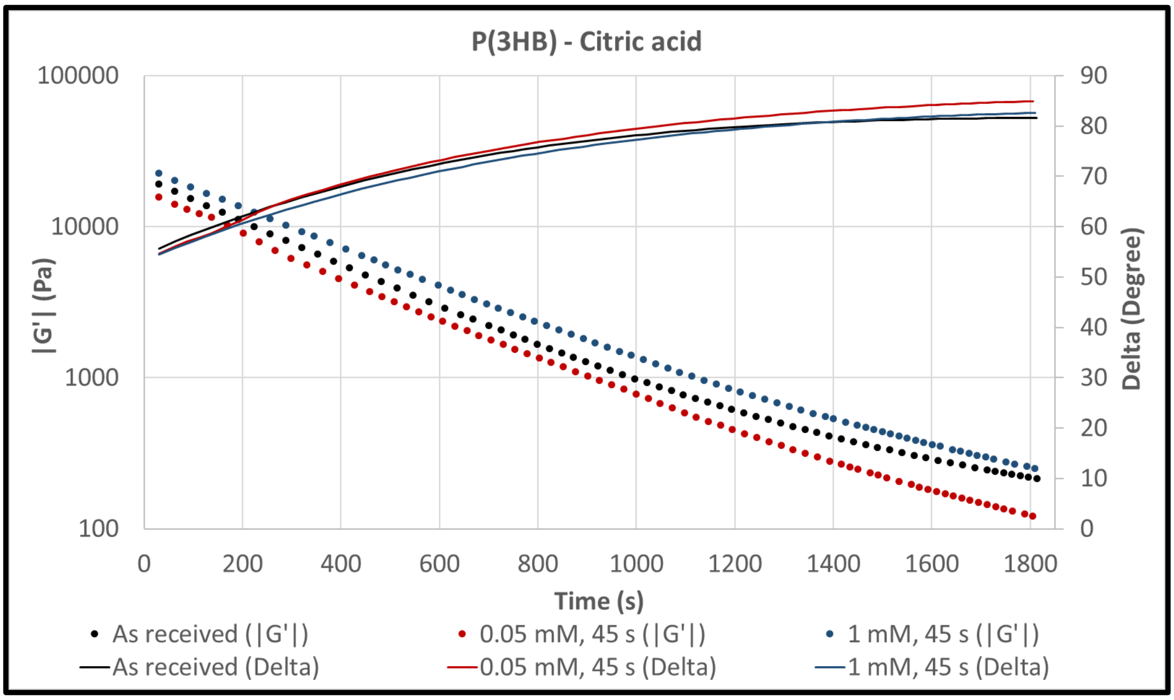


Figure 50. The modulus and delta curves for the three different samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s. The modulus and delta is shown as a function of time.

The results from these rheometer runs showed that there was not a big difference between the samples. This corresponds well to the TGA results that state that there was no temperature difference between the washed samples.

4.4.2 P(3,4HB)

4.4.2.1 TGA

The temperatures received from the different washing times for two different acid concentrations are shown in Table 23. For this biopolymer, a lower degradation temperature was obtained for the sample washed with 0.05 mM citric acid, and a longer washing time would not improve the result. The only way to achieve a higher degradation temperature was to increase the citric acid concentration. While doing that, a longer washing time had no effect on the degradation temperature.

Table 23. The temperature received from different washing times (45 s, 5 min, 30 min and 2 h) for two different acid concentrations (0.05 mM and 1.00 mM).

	45 s	5 min	30 min	2 h
0.05 mM	274℃	274℃	274℃	274℃
1.00 mM	296℃	296℃	295℃	296℃

4.4.2.2 DSC

Also in this case, 45 seconds was enough washing time for both the concentrations. Therefore, only samples with a washing time of 45 seconds were further investigated.

Measurements of unprocessed powder and powder processed in the rheometer were made to see how the biopolymer properties changed after being subjected to heat and movement.

For the copolymer, two melting temperatures (T_m) and two crystallization temperatures (T_c) was obtained. The two melting temperatures corresponds to a melting-recrystallization-remelting process. T_c corresponds to the crystallization temperature received when the sample was crystallized during cooling. In this case, not all the sample was able to crystallize during the cooling, so the rest of the sample crystallized when heated again (T_{cc}), called cold crystallization. Also, a glass transition temperature (T_g) was found. ΔH_m stands for the enthalpy required to melt the sample, and ΔH is the enthalpy required to crystallize the sample (ΔH_c during cooling and ΔH_{cc} during heating).

4.4.2.2.1 Unprocessed Powder

Table 24 shows different parameters received from the DSC.

Table 24. Parameters received form the DCS for three unprocessed samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s.

	$T_{m,1}$ (℃)	$T_{m,2}$ (℃)	T_c (℃)	T_{cc} (℃)	T_g (℃)	ΔH_m (J/g)	ΔH_c (J/g)	ΔH_{cc} (J/g)	X_c (%)
As-received	131	149	49	41	-1.9	69	13	27	47
0.05 mM, 45 s	147	160	83	46	2.6	71	48	2.0	49
1.00 mM, 45 s	145	159	79	38	1.7	73	35	10	50

Figure 51 shows the heating and cooling cycles for the as-received sample, and for the samples washed in 0.05 and 1000 mM citric acid for 45 seconds.

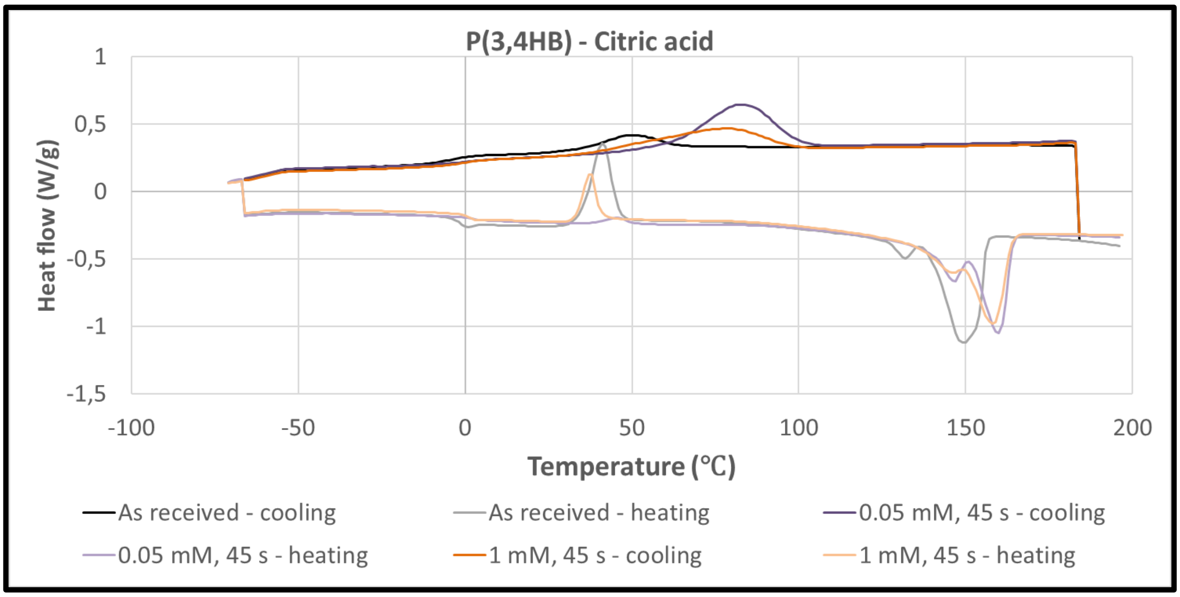


Figure 51. The heating and cooling curves from three different samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s. The heat flow is plotted as a function of temperature.

These results follow the other DSC results well. The melting temperatures were higher for the acid washed samples. The crystallization temperatures for the acid washed samples were almost the same, and higher than for the as received sample. The glass transition temperature was higher for the acid washed samples. The melting enthalpies are in the same range for all the samples. The crystallization enthalpies were higher for the acid washed samples compared to the as received one, followed by a lower cold crystallization enthalpy for the acid washed sample.

4.4.2.2.2 Processed Powder

Table 25 shows the properties of the biopolymer after been processed in the rheometer.

Table 25. Different parameters received form the DCS for three processed samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s.

	$T_{m,1}$ (°C)	$T_{m,2}$ (°C)	T_c (°C)	T_{cc} (°C)	T_g (°C)	ΔH_m (J/g)	ΔH_c (J/g)	ΔH_{cc} (J/g)	X_c (%)
As-received	107	125	-	57	-15	48	-	45	33
0.05 mM, 45 s	146	157	99	-	1.5	78	67	-	53
1.00 mM, 45 s	155	165	110	-	3.2	71	63	-	49

By comparing the degree of crystallinity for P(3HB) and P(3,4HB), it can be seen that it was higher for P(3HB). This seemed likely because P(4HB) is an amorphous polymer and tends to decrease the crystallinity when working as a copolymer with P(3HB), who is a semi-crystalline polymer.

Table 25 is based on the heating- and cooling cycles in Figure 52.

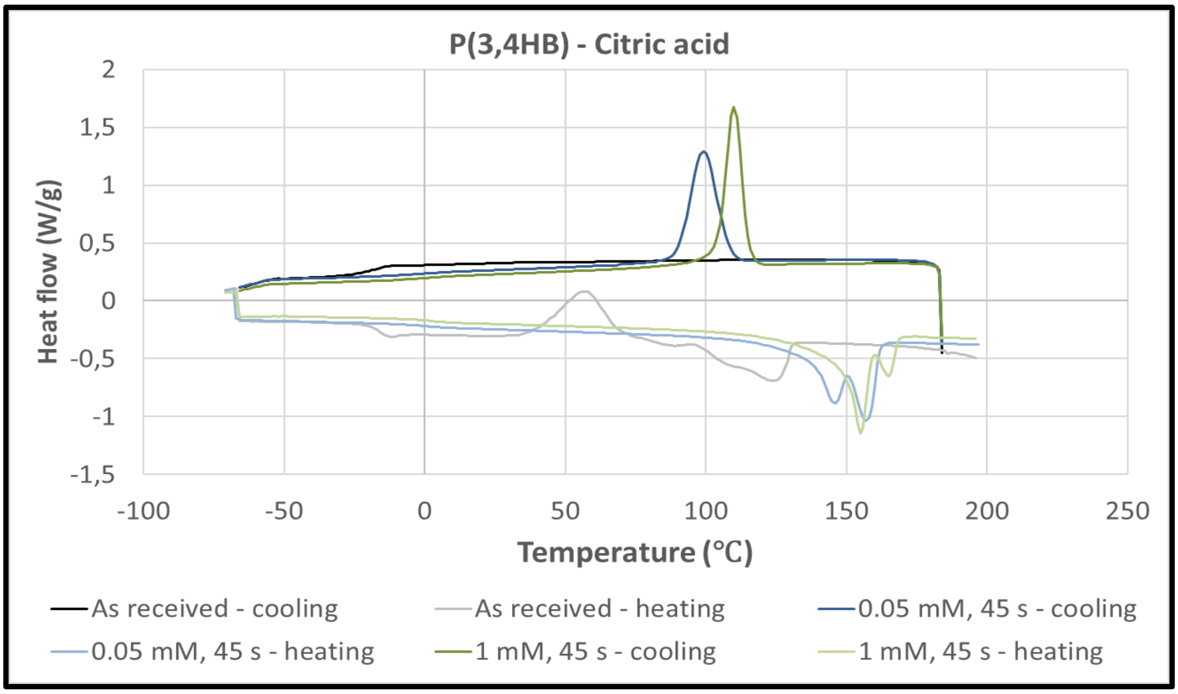


Figure 52. The DSC heating and cooling curves for three different samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s. The heat flow is shown as a function of temperature.

Both $T_{m,1}$ and $T_{m,2}$ were higher for the acid washed samples. No crystallization temperature existed for the as-received sample, but for the acid washed samples, the crystallization temperature was increased with increasing acid concentration. No cold crystallization temperatures existed for the acid washed samples. The glass transition temperature for the as-received sample was significant lower than for the acid washed samples. The melting enthalpies were almost the same for the acid washed samples, and higher than for the as-received sample. No crystallization enthalpy existed for the as-received sample, and no cold crystallization enthalpies for the acid washed samples.

4.4.2.3 Rheometer

The result from the rheometer is shown in Figure 53.

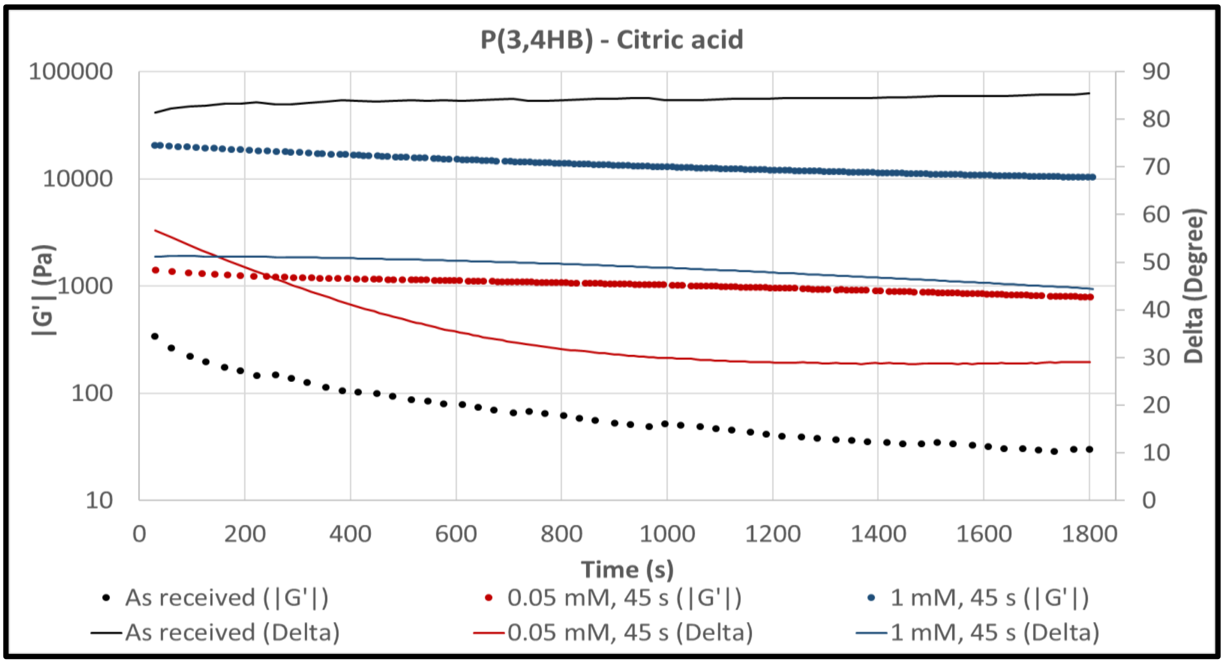


Figure 53. The modulus and delta curves from different copolymer samples; as-received, 0.05 mM 45 s, and 1.00 mM 45 s, are plotted as a function of time.

These rheometer results showed that the as-received sample had the lowest modulus which means that this sample was the most unstable. Even though the curves for the two washed samples lie apart from each other, they had almost the same slope which indicates that they had a similar stability, and they were also more stable than the as-received sample.

Since the as-received sample had the highest delta curve that increases with time, it was more unstable because the viscosity became lower. Also here, the curves from the two washed samples were located apart from each other but had not the same slope. The delta curve for 1 mM, 45 s sample was very stable throughout the measurement, while for the 0.05 mM, 45 s sample the delta curve decreases in the beginning and was stabilized at the end.

4.5 Part 4 – Comparing Unprocessed and Processed Samples

In this part, comparison between unprocessed and processed samples has been made to see how the biopolymers are affected when subjected to heat and movement in the rheometer.

The results from TGA, DSC and SEC analysis of P(3HB) and P(3,4HB) are shown in Figure 54-64, and Table 26-29.

4.5.1 P(3HB)

4.5.1.1 TGA

The results from the TGA measurements of the unprocessed and processed homopolymer P(3HB) are compared in Table 26.

Tabell 26. Comparison between degradation temperatures for unprocessed and processed P(3HB) samples; as-received, 0.05 mM 30 min, 1000 mM 30 min, 0.05 mM 45 s, and 1.00 mM 45 s.

	$T_{Unprocessed}$ (℃)	$T_{Processed}$ (℃)
As-received	279	285
0.05 mM, 30 min	296	294
1000 mM, 30 min	294	295
0.05 mM, 45 s	295	296
1.00 mM, 45 s	294	296

As Table 26 shows, there was almost no difference between the unprocessed and processed citric acid washed samples. The only difference obtained was between the as-received samples. The degradation temperature was higher for the sample that has been processed in the rheometer. This result was a bit strange since the processed sample has been exposed to mechanical processing in the rheometer, and this would more likely lead to a degradation of polymer chains and further a less stable sample.

4.5.1.2 DSC

The DSC results of the unprocessed and processed homopolymer are shown in Figure 54-58.

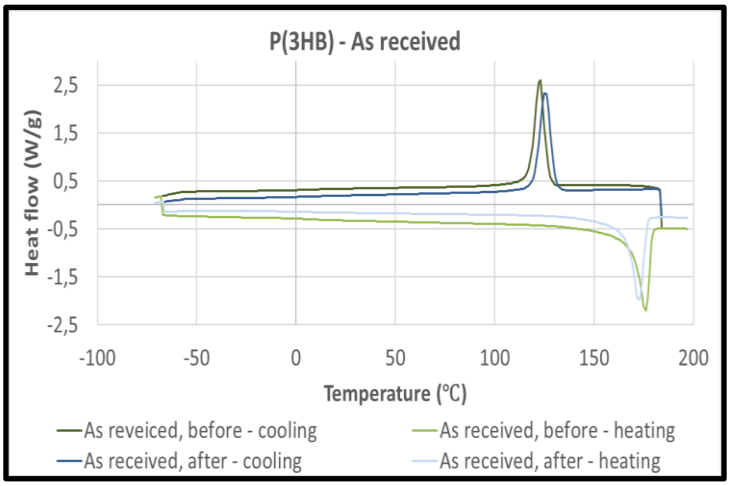


Figure 54. As-received sample before and after processing in the rheometer.

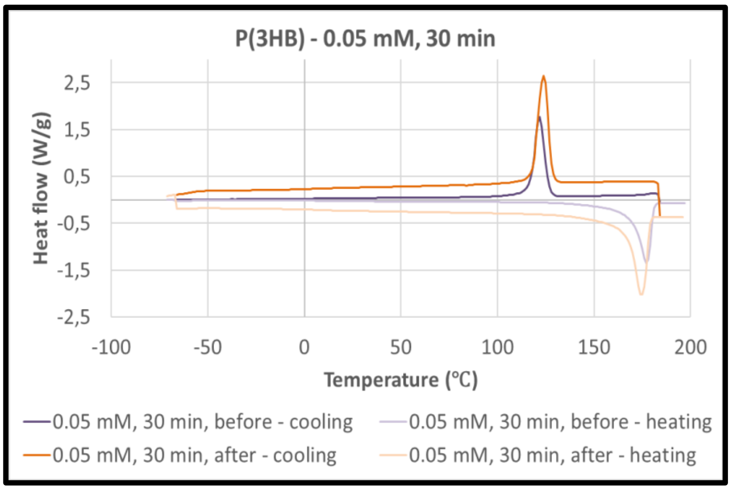


Figure 55. 0.05 mM, 30 min sample before and after being processed in the rheometer.

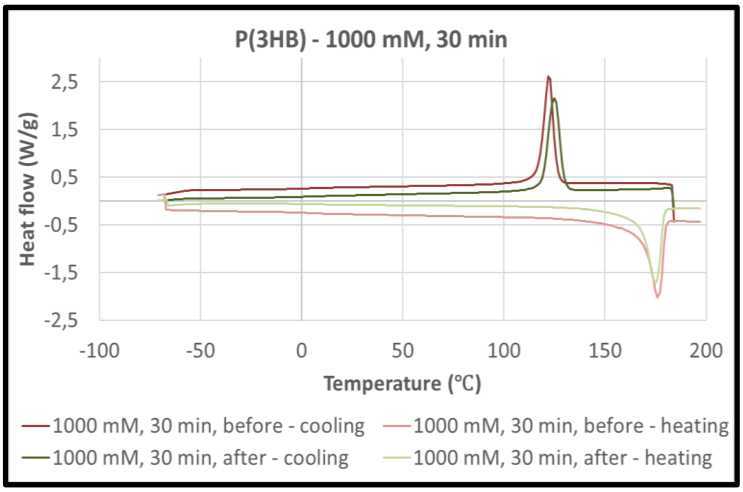


Figure 56. 1000 mM, 30 min sample before and after being processed in the rheometer.

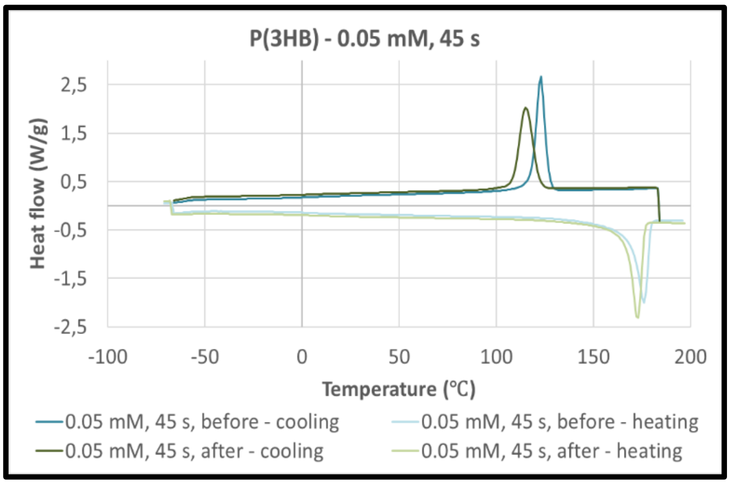


Figure 57. 0.05 mM, 45 s sample before and after being processed in the rheometer.

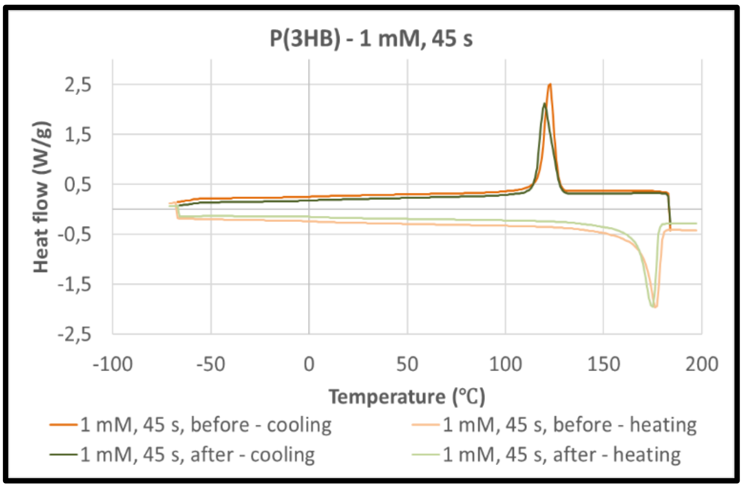


Figure 58. 1.00 mM, 45 s sample before and after being processed in the rheometer.

The comparison between all the unprocessed and processed samples showed a minor difference in the curves from the DSC. This should mean that the samples were not degraded significant in the rheometer and retained their properties.

4.5.1.3 SEC

The weight- and number molecular weights from the SEC measurement can be seen in Table 27.

Table 27. Comparison of the number and weight molecular weights, and the DPI between different unprocessed and processed samples.

	M_w (g/mole)	M_n (g/mole)	PDI
Unprocessed, as-received, powder	85 600	60 900	1.4
Processed, as-received, powder	106 600	70 800	1.5
Processed, 0.05 mM, 30 min	110 000	77 500	1.4
Processed, 1000 mM, 30 min	125 900	83 900	1.5
Processed, 0.05 mM, 45 s	202 400	126 000	1.6
Processed, 1.00 mM, 45 s	125 000	75 800	1.6

The molecular weights received were somewhat contradicting. The as-received, processed powder should have the lowest molecular weight because it was not washed with citric acid and are therefore less thermally stable. However, the unprocessed powder should have a higher molecular weight than the processed powder because the molecular chains have not been subjected to any heat or movement from the rheometer.

The 0.05 mM, 45 s sample had the highest molecular weight by far. This result does not match the TGA results which said that the 0.05 mM, 30 min sample had the highest degradation temperature (296°C). The other three washed samples had a degradation temperature at 294°C which should mean that all the four samples should have molecular weights close to each other.

Why the SEC results are contradicting was further investigated in part 8.

4.5.2 P(3,4HB)

4.5.2.1 TGA

The results from the TGA measurements of the unprocessed and processed copolymer are compared in Table 28.

Tabell 28. Comparison between degradation temperatures for unprocessed and processed P(3,4HB) sample; as-received, 0.05 mM 30 min, 0.25 mM 30 min, 1.00 mm 30 min, 0.05 mM 45 s, and 1.00 mM 45 s.

	$T_{Unprocessed}$ (℃)	$T_{Processed}$ (℃)
As-received	247	246
0.05 mM, 30 min	274	274
0.25 mM, 30 min	281	281
1 mM, 30 min	295	296
0.05 mM, 45 s	274	275
1.00 mM, 45 s	296	294

The values from Table 28 show no difference in degradation temperatures between the unprocessed and processed samples. This result might depend on that the samples were not degraded in the rheometer and therefor retained their properties.

4.5.2.2 DSC

The DSC results from the unprocessed and processed copolymers are compared in Figure 59-64.

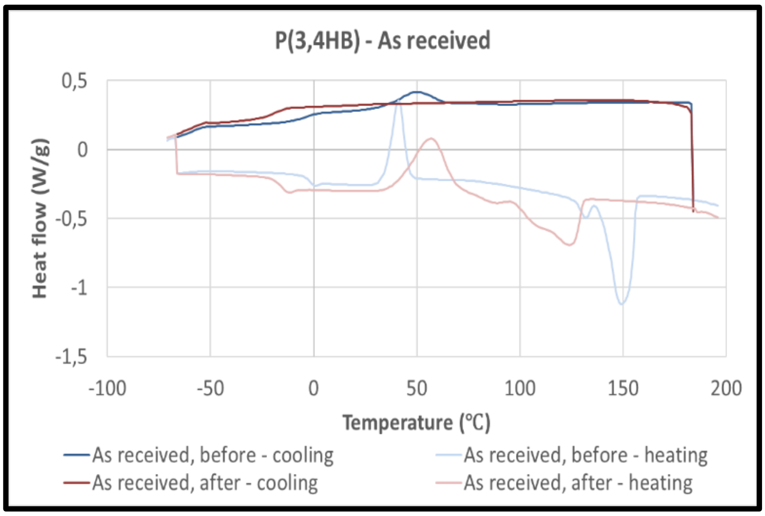


Figure 59. As-received sample before and after being processed in the rheometer.

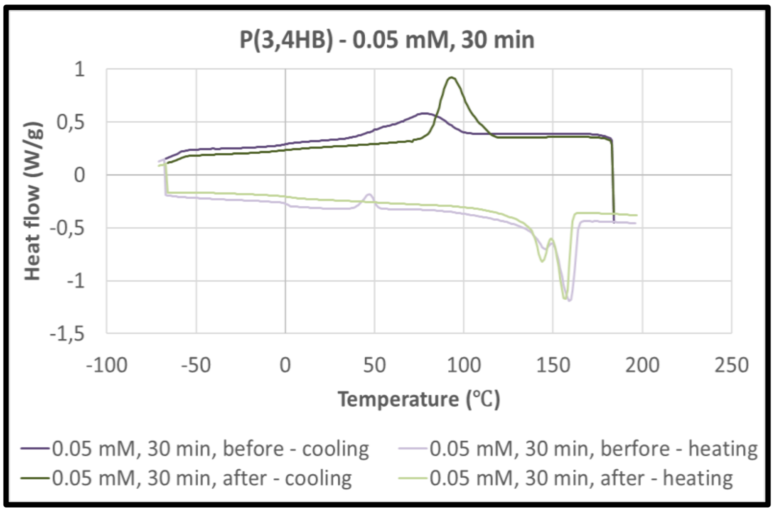


Figure 60. 0.05 mM, 30 min sample before and after being processed in the rheometer.

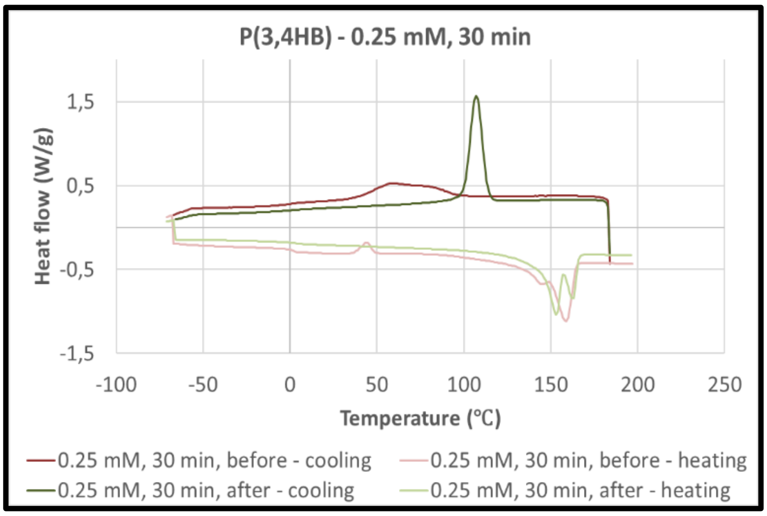


Figure 61. 0.25 mM, 30 min sample before and after being processed in the rheometer.

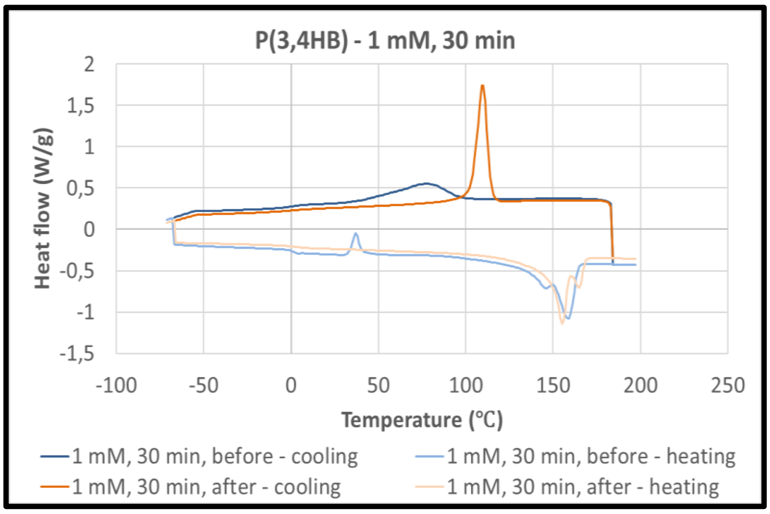


Figure 62. 1 mM, 30 min sample before and after being processed in the rheometer.

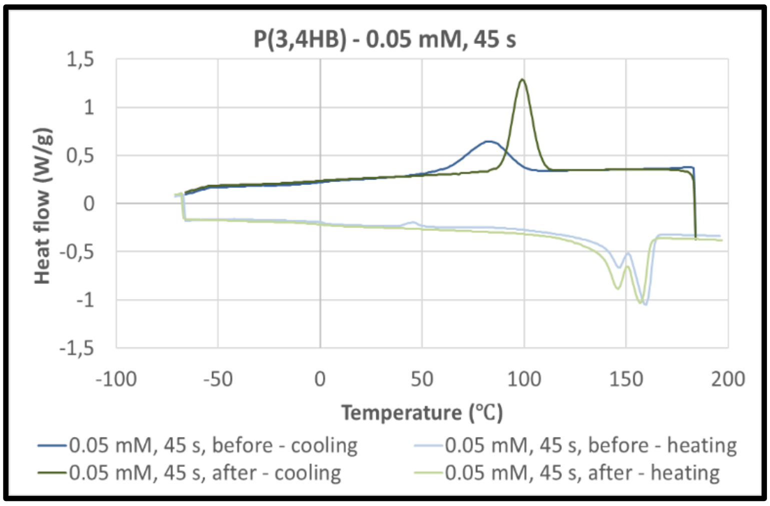


Figure 63. 0.05 mM, 45 s sample before and after being processed in the rheometer.

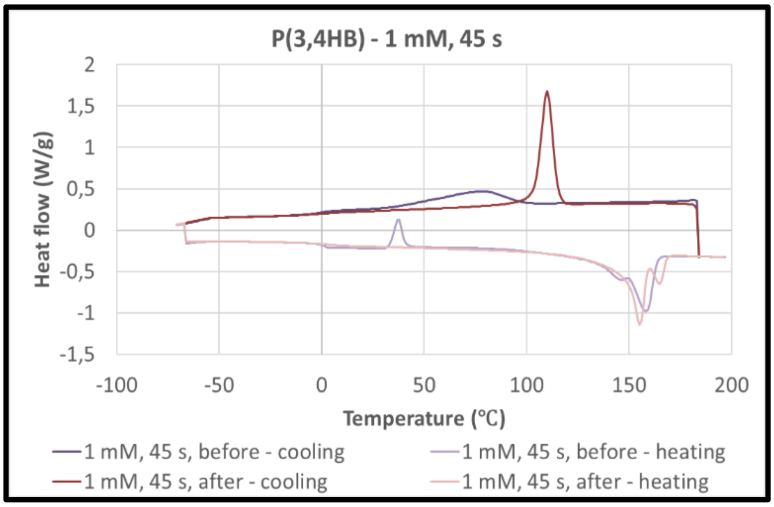


Figure 64. 1 mM, 45 s sample before and after being processed in the rheometer.

For P(3,4HB), there was a big difference between the curves received before and after processing. This especially for the cooling curves. After the samples have been processed, they crystallize totally during cooling, except for the as-received sample.

This difference may depend on the fact that a temperature of 185℃ was used to erase the thermal history for the copolymer as well. Since the melting temperature of P(3,4HB) is 170℃, a temperature of 185℃ may be too high above the melting temperature. A temperature of 175℃ should have been used instead. This was further investigated in part 8.

4.5.2.3 SEC

The weight- and number molecular weights from the SEC measurement can be seen in Table 29.

Table 29. Comparison of the number and weight molecular weights, and the PDI between different processed and unprocessed samples.

	M_w (g/mole)	M_n (g/mole)	PDI
Unprocessed, as-received, powder	Poor curves	Poor curves	-
Processed, as-received, powder	Unable to dissolve	Unable to dissolve	-
0.05 mM, 30 min	39 500	21 400	1.8
0.25 mM, 30 min	70 500	46 400	1.6
1 mM, 30 min	145 700	100 200	1.5
0.05 mM, 45 s	33 000	17 700	1.9
1.00 mM, 45 s	112 100	76 800	1.5

For the copolymer, there was no result for the two as-received powders. The unprocessed powder gave curves that were too poor to be analyzed. For the processed powder, the peace of plastic was not able to dissolve in chloroform and an analyze could therefore not be performed. Why the sample of processed as-received powder could not be dissolved in chloroform might depend on the fact that a network was created in the material during the rheometer analysis.

Otherwise, the TGA and SEC results corresponds well to each other. The TGA result showed that the two 1 mM samples had the highest thermal stability, and the two 0.05 mM samples had the lowest thermal stability, which corresponds well to the SEC result. A higher degradation temperature means that the sample had a higher thermal stability and the molecular chains have not broken down that much when processed.

A further investigation of the reliance of the SEC results was made in part 8.

4.6 Part 5 – Formic Acid

Formic acid was also chosen for analyzing. This to be able to compare the results from citric acid with one other acid. Table 30 – 31 shows the comparison between the degradation temperatures for both P(3HB) and P(3,4HB).

Formic acid was only analyzed with TGA.

4.6.1 P(3HB)

Table 30 compares the degradation temperatures for the homopolymer samples washed with citric acid and formic acid.

Table 30. A comparison between the degradation temperatures for citric acid and formic acid for as-received, 0.05 mM, 1.00 mM and 1000 mM P(3HB) samples.

	Citric acid	Formic acid
As-received	279°C	279°C
0.05 mM	296°C	296°C
1.00 mM	296°C	296°C
1000 mM	294°C	296°C

In Table 30, one can see that there was almost no difference in the degradation temperature between the two acids

4.6.2 P(3,4HB)

Table 31 compares the degradation temperatures for the copolymer samples washed with citric acid and formic acid.

Table 31. A comparison between the degradation temperatures for citric acid and formic acid for as-received, 0.05 mM, 1.00 mM and 1000 mM P(3,4HB) samples.

	Citric acid	Formic acid
As-received	247°C	247°C
0.05 mM	274°C	273°C
1.00 mM	295°C	290°C
1000 mM	295°C	297°C

For citric acid, there was a 27°C-temperature difference between the as-received powder and the 0.05 mM sample. The degradation temperature was further increased when 1 mM citric acid is used. For formic acid, the same increase in the degradation temperature as for citric acid was obtained when washing with a concentration of 0.05 mM. The highest degradation temperature was however reached when washing with 1000 mM.

4.6.3 Common conclusion

Since the two acids gave approximately the same results, but formic acid has less desirable properties than citric acid, the decision to only proceed with citric acid was made.

4.7 Part 6 – As-received Powder mixed with Citric Acid

Part 6 was analyzed with TGA. The results are shown in Table 32-33, and Figure 65-66.

4.7.1 P(3HB)

Table 32 shows the degradation temperature for the as-received powder with citric acid, as-received powder, and powder washed with 1 mM citric acid.

Table 32. Degradation temperatures for three different samples; as-received powder mixed with citric acid, as-received and citric acid.

	As-received powder mixed with citric acid	As-received	Citric acid
Degradation temperature (°C)	252	279	296

Figure 65 shows the weight percent of the three samples as a function of temperature.

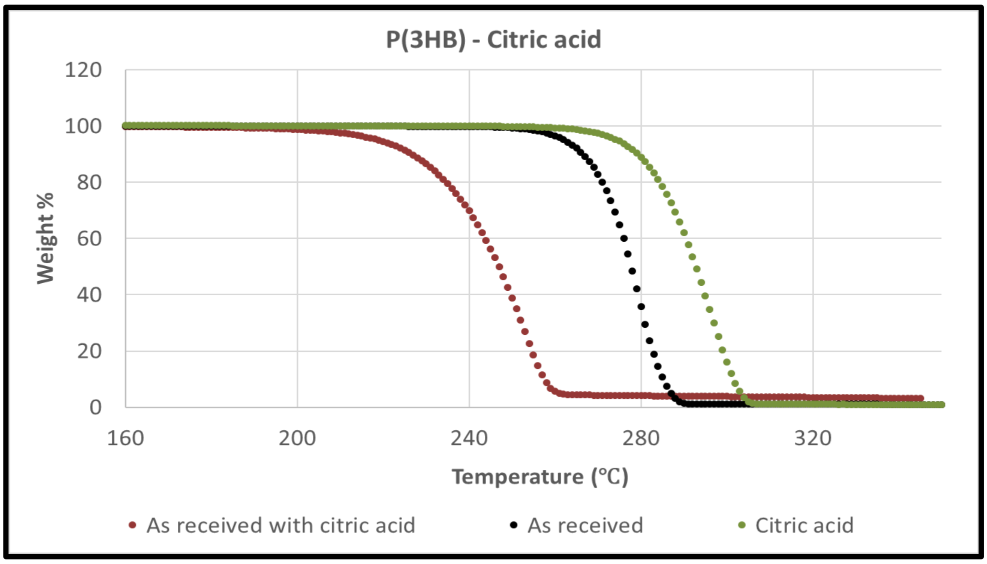


Figure 65. The weight percent as a function of temperature for three P(3HB) samples; as-received powder mixed with citric acid, as- received and citric acid. The degradation temperature is obtained at the highest slope.

There was a significant difference in the degradation temperature between the as-received powder, powder washed with 1 mM citric acid for 30 minutes, and as-received powder mixed with citric acid. This clearly showed that the citric acid added to the powder must be washed away thoroughly or the degradation temperature would be lowered even more.

4.7.2 P(3,4HB)

Table 33 shows the degradation temperature for the as-received powder mixed with citric acid, as-received powder, and powder washed with 1 mM citric acid.

Table 33. The degradation temperature for three different samples; as-received powder mixed with citric acid, as-received and citric acid.

	As-received powder mixed with citric acid	As-received	Citric acid
Degradation temperature (°C)	250	247	295

Figure 66 shows the weight percent of the three samples as a function of temperature.

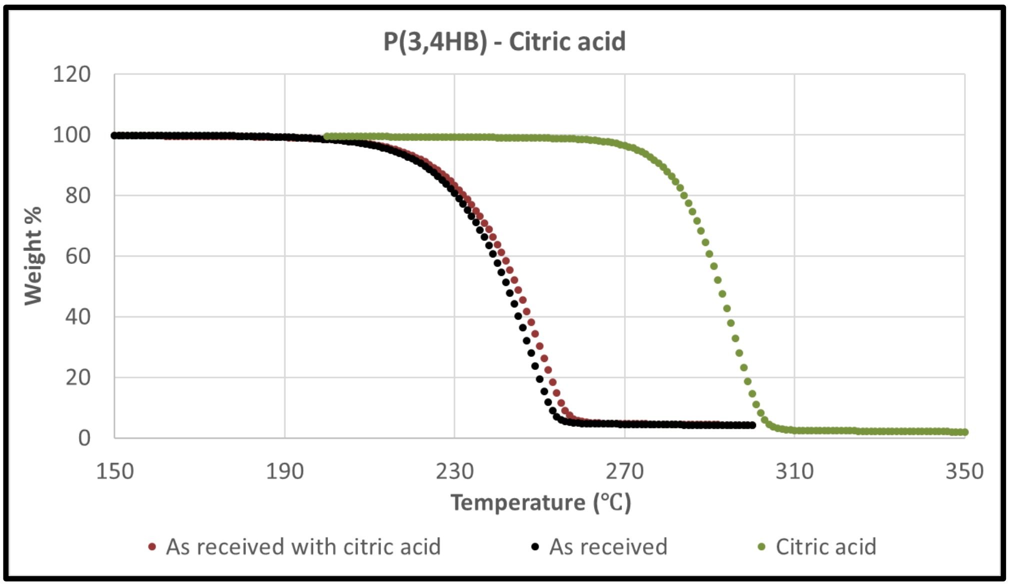


Figure 66. The weight percent as a function of time for three P(3HB) samples; as-received powder mixed with citric acid, as- received and citric acid. The degradation temperature is obtained at the highest slope.

For the copolymer, the as-received sample with citric acid was almost the same as the as-received. Here, deterioration was not seen as for the homopolymer. Since the degradation temperature for the as-received sample and the as-received with citric acid sample was almost the same, this might depend on that no citric acid was present in the selected sample. The amount of citric acid mixed into the powder was very small, so it was impossible to know before the analyze, if the selected sample contained citric acid or not.

4.8 Part 7 – Water Wash

In this part, the samples were evaluated with TGA. The results are shown in Table 34-37 and Figure 67-68.

4.8.1 P(3HB)

4.8.1.1 Step 1

Table 34 shows the degradation temperature for the sample washed with only water for 30 minutes, the as-received sample, and sample washed with 1 mM citric acid.

Table 34. The degradation temperatures for three different samples; as-received powder, powder washed in deionized water for 30 min, and powder washed with 1 mM citric acid solution.

	As-received	Water (30 min)	Citric acid
Degradation temperature (℃)	279	294	296

The data in Table 34 were obtained from Figure 67, where the weight percent was plotted as a function of temperature.

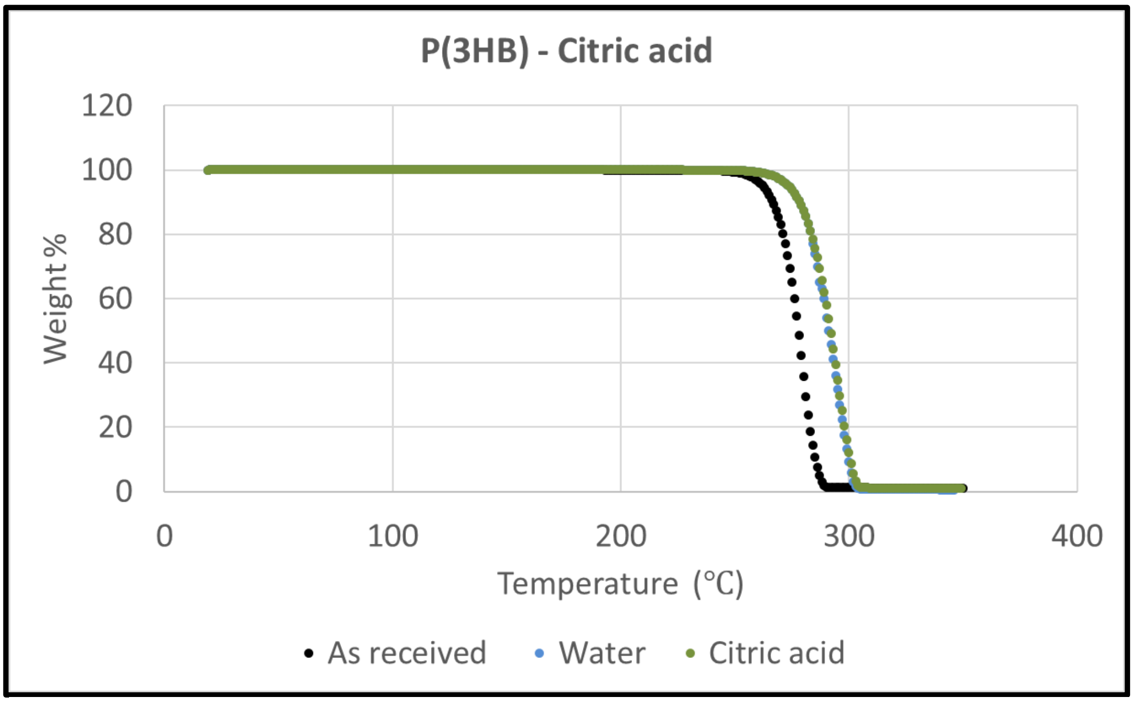


Figure 67. The weight % as a function of temperature for three different samples; as-received powder, powder washed in deionized water for 30 min, and powder washed with 1 mM citric acid solution. The degradation temperature is obtained at the highest slope.

The result in Figure 67 might be the most interesting result so far in the experiment. Washing the homopolymer with only water gave almost the same result as washing with citric acid. Only a 2℃ difference was obtained.

4.8.1.2 Step 2

This was investigated further with three more washing times; 45 seconds, 5 minutes, and 2 hours. All the degradation temperatures from step 1 and 2 are shown in Table 35.

Table 35. Degradation temperatures for samples washed with different washing times together with the degradation temperatures for the as-received sample and the sample washed with citric acid.

	As-received	45 s	5 min	30 min	2 h	Citric acid
Degradation temperature (℃)	279	296	296	294	294	296

As can be seen in the table above, no significant difference in temperature was obtained between the different washing times. This experimental part shows that it was almost enough to wash the homopolymer with water to reach the highest obtained degradation temperature.

4.8.2 P(3,4HB)

4.8.2.1 Step 1

Table 36 shows the degradation temperatures for the sample washed with only water, the as-received sample, and sample washed with 1 mM citric acid.

Table 36. The degradation temperatures for three different samples; as-received powder, powder washed in deionized water for 30 min, and powder washed with 1 mM citric acid solution.

	As-received	Water (30 min)	Citric acid
Degradation temperature (℃)	247	271	295

The data in Table 36 were obtained from Figure 68, where the weight percent is plotted as a function of temperature.

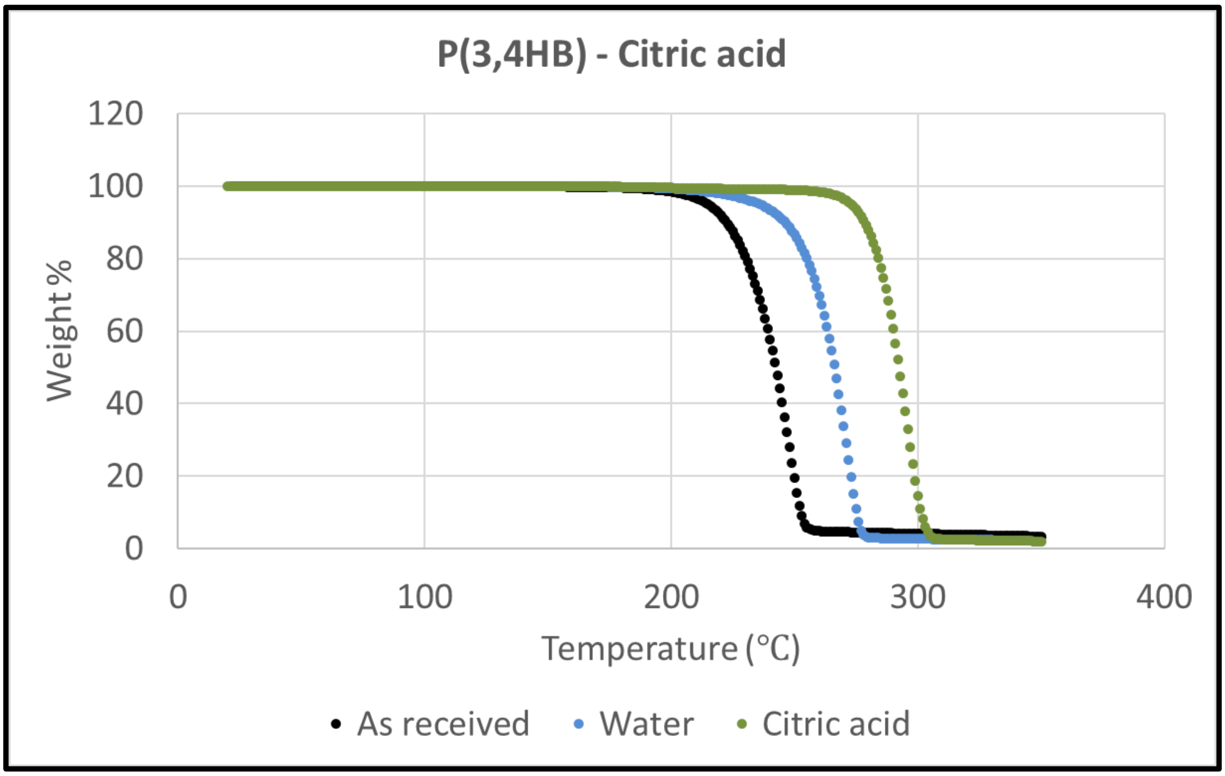


Figure 68. The weight % as a function of temperature for three different samples; as-received powder, powder washed in deionized water for 30 min, and powder washed with 1 mM citric acid solution.

The copolymer did not reach as high degradation temperature as the homopolymer. The thermal stability was improved, but not enough to compete with the acid wash. This corresponds well to the results in part 2 that a higher degradation temperature for the copolymer was harder to reach than for the homopolymer.

4.8.2.2 Step 2

Three more washing times were studied; 45 seconds, 5 minutes, and 2 hours. All the degradation temperatures from step 1 and 2 are shown in Table 37.

Table 37. Degradation temperatures from four different washing times (45 s, 5 min, 30 min and 2 h), along with the as received, and with citric acid.

	As-received	45 s	5 min	30 min	2 h	Citric acid
Degradation temperature (°C)	247	268	271	271	270	295

For the copolymer, the water wash did not gave as high degradation temperature as the citric acid wash (15°C difference). It does however gave a higher degradation temperature compared to the as-received powder (~23°C difference). The results in this experimental part showed that a citric acid wash gave the highest degradation temperature.

4.9 Part 8 – Error Sources

To conclude if the SEC and DSC gave reliable results, an investigation of possible error sources was made.

4.9.1 SEC

4.9.1.1 Calculation of the molecular weight

It was of interest to see if the calculations of the weight- and number molecular weights on the computer gave the same results when repeated. It was in most cases hard to establish where the base line of the peak should start and end, this may depend on weak signals from the equipment because the amount of sample injected in the SEC was very small. To change the amount of sample injected was not possible. The arrows in Figure 69 below show three possible starting points of the curve.

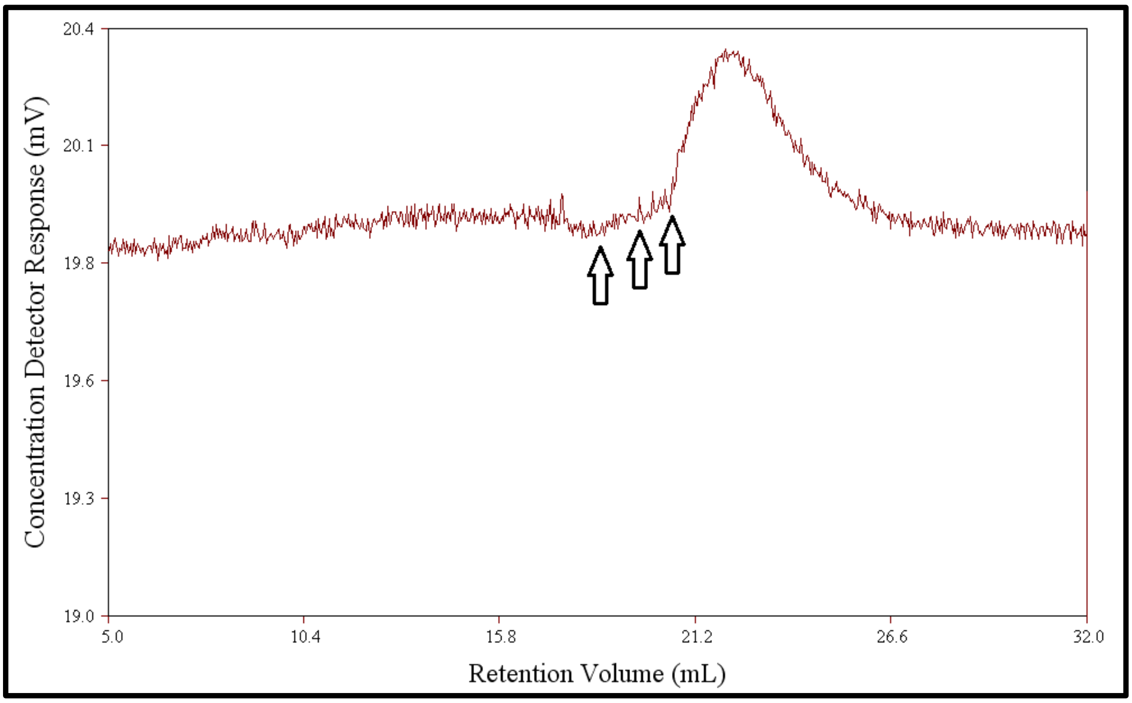


Figure 69. The arrows show possible options where to begin with the baseline of the peak while calculating the molecular weight in the program. Here, the human factor will result in different molecular weights.

The molecular weight calculations were made two times for each sample, by the same person, to see if the results would differ. The results can be seen in Table 38 and 39. The two values in each box stands for the first and the second calculation.

4.9.1.1.1 P(3HB)

Table 38. Comparison of two molecular weight calculations made for the same samples for both the number and weight molecular weight.

	M_w (g/mole)	M_n (g/mole)
Unprocessed, As-received powder	85 600 / 86 700	60 900 / 67 000
Processed, As-received powder	106 600 / 110 700	70 800 / 81 500
Processed, 0.05 mM, 30 min	110 000 / 103 100	77 500 / 57 200
Processed, 1000 mM, 30 min	125 900 / 132 800	83 900 / 101 900
Processed, 0.05 mM, 45 s	202 400 / 193 600	126 000 / 137 800
Processed, 1.00 mM, 45 s	125 000 / 112 100	75 800 / 62 400

4.9.1.1.2 P(3,4HB)

Table 39. Comparison of two molecular weight calculations made for the same samples for both the number and weight molecular weight.

	M_w (g/mole)	M_n (g/mole)
Unprocessed, As-received powder	Poor curves	Poor curves
Processed, As-received powder	Unable to dissolve	Unable to dissolve
Processed, 0.05 mM, 30 min	38 100 / 39 500	25 000 / 21 400
Processed, 0.25 mM, 30 min	69 900 / 70 500	45 800 / 46 400
Processed, 1.0 mM, 30 min	148 000 / 145 700	101 000 / 100 200
Processed, 0.05 mM, 45 s	33 000 / 29 200	17 700 / 16 200
Processed, 1.00 mM, 45 s	32 000 / 112 100	17 400 / 76 800

As can be seen in Table 38 and 39, there was a difference in the molecular weights for the exact same curve. This error was most likely due to the human factor and will therefore be hard to overcome. For most samples, the difference between the values was not that alarming. The only sample that really stands out was 1 mM, 45 s, for P(3,4HB), where the difference between the two calculation was unreasonable. Over all, the calculations made by hand seem reliable.

4.9.1.2 Plastic pieces selected from the sample

Another error source that most likely affected the results had to do with the plastic pieces picked out for analyze. Depending on which piece that was selected, the properties of the material could vary. Different parts of the melted sample form the rheometer may not have been subjected to the same heat and motion. The polymer chains on the surface of the tablet may have been processed harder than the polymer chains in the middle of the tablet. At the surface, the sample was subjected to more motion than the more protected part of the sample in the middle. Therefore, the processed sample obtained different properties at different places and this may have been a major error source for the samples analyzed after being processed.

A way to avoid this problem would be to mix the processed sample into a homogeneous mix before further analyze. Unfortunately, there was not enough time to carry out the mixing and redo the following analyzes.

4.9.2 DSC

4.9.2.1 Comparison between two different plastic pieces

Which part from the sample that was selected after being processed in the rheometer may also affect the DSC analyze. Figure 70 shows two cycles where the samples come from two different places of the plastic piece showed in Figure 28.

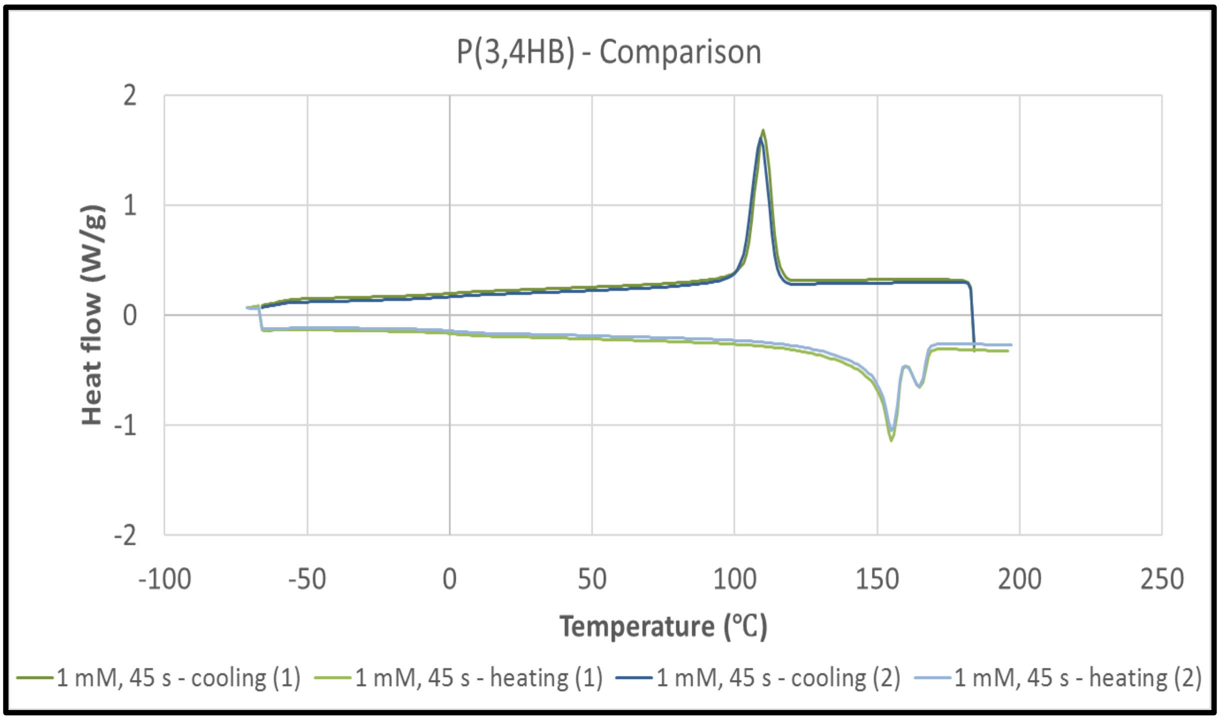


Figure 70. DSC cooling and heating cycles of the same sample but with different pieces of processed plastic.

As can be seen in the Figure 70 above, the two curves matched very well, which was a bit strange. One reason for this was that even if two pieces were selected, they may have been processed in the same way. It was impossible to see how the sample was located in the rheometer after running, so even if a new piece was selected this did not mean that it had been processed in a different way compared to the first piece. Another explanation why the cycles are so much alike was that there was no significant property difference between the different parts in the processed sample.

4.9.2.2 Different heating temperatures for P(3,4HB)

As mentioned in the theory for DSC, the thermal history for the sample had to be removed before the valid measurement could take place. This was done by raising the temperature to 185°C for both the biopolymers. P(3HB) has a melting temperature of 180°C so a temperature of 185°C seemed suitable to be sure that the whole sample was melted. But since P(3,4HB) has a melting temperature of around 170°C, a temperature of 185°C might be too high, and the material starts to degrade to much during the heating. This might affect the sample in a way that the properties were being changed to much before the interesting analyze takes place. To see what happens with the sample when not heating the sample to 185°C, new analyzes were performed where the temperature was set to 175°C instead. This was done for both unprocessed and processed P(3,4HB). The DSC results can be seen in Figure 71 and 72 below.

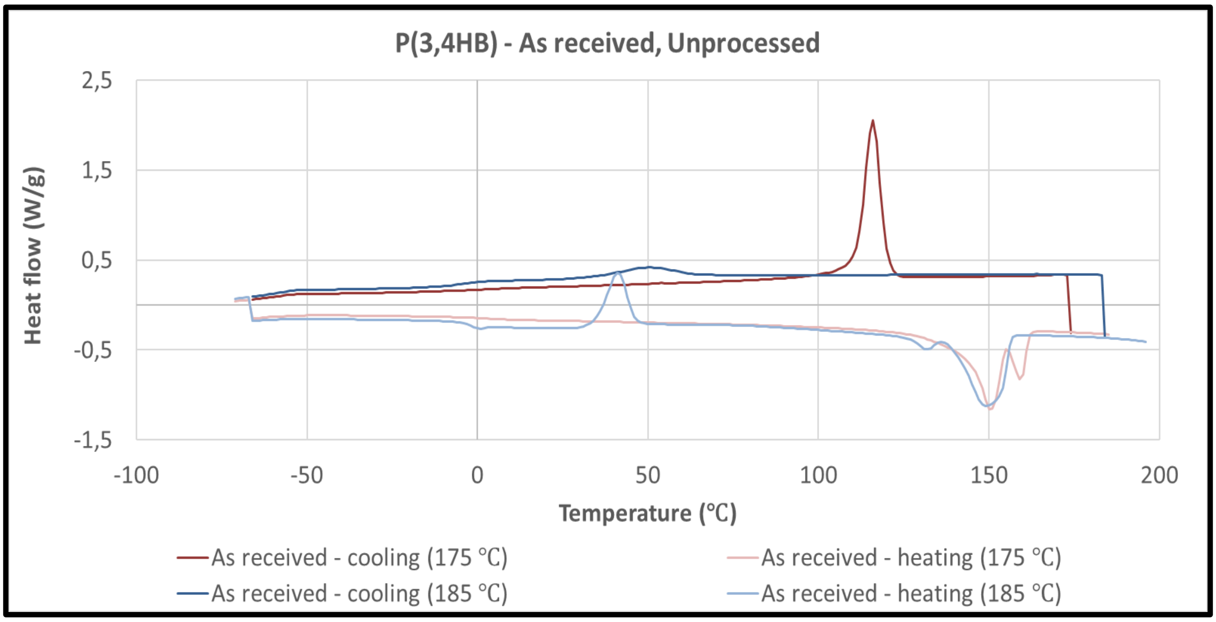


Figure 71. Unprocessed, as-received P(3HB) sample with different temperatures to erase the thermal history of the sample; 175°C and 185°C. This to see how different temperatures would affect the crystallization and melting of the sample.

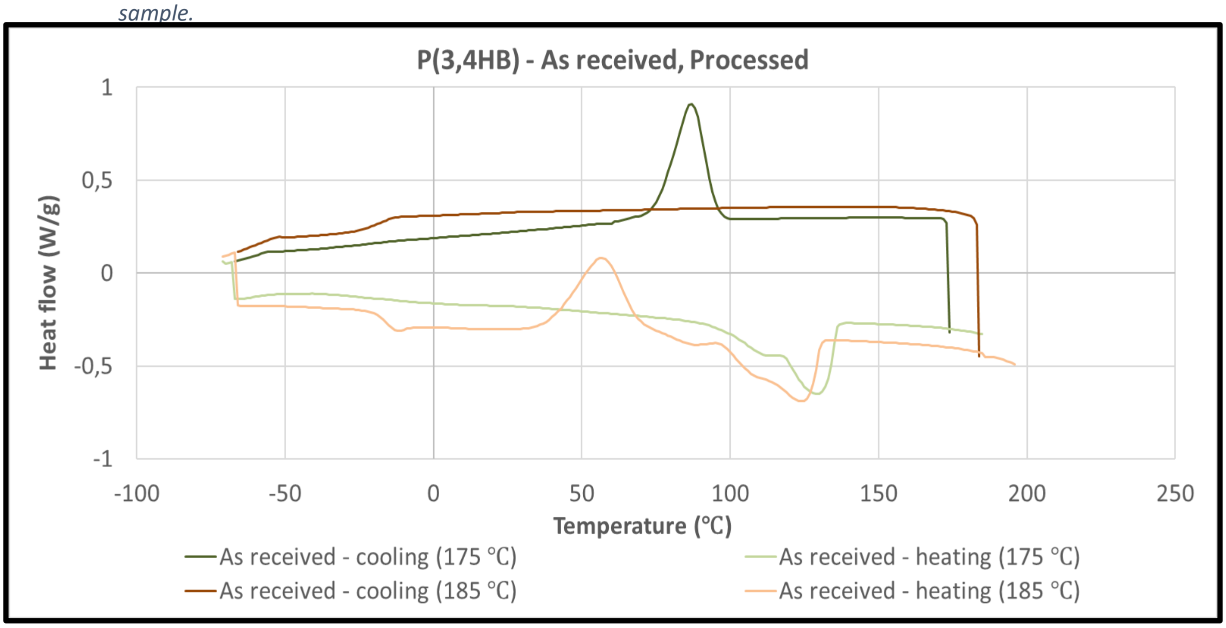


Figure 72. Processed, as-received P(3,4HB) sample with different temperatures to erase the thermal history of the sample; 175°C and 185°C. This to see how different temperatures would affect the crystallization and melting of the sample.

As can be seen in Figure 71 and 72, there was a big difference between the samples when they are being heated to 175°C and 185°C. For both unprocessed and processed powder, there was almost no crystallization obtained during cooling when the samples were heated to 185°C. The crystallization occurs instead during heating. When the samples were heated to 175°C, both samples completely crystallize when being cooled and there was no cold crystallization present. A glass transition temperature was only obtained for the samples being heated to 185°C.

When the biopolymer was heated to 185°C, the chains were degraded to a higher extent which seems to make it more difficult for the polymer to crystallize during cooling.

5 Conclusion

The experiments in the first part showed that citric acid gave the highest degradation temperature for both the biopolymers. Therefore, this acid was further investigated with different concentrations and washing times. For P(3HB), a concentration of 0.05 mM and a washing time of 45 s was enough to give the highest degradation temperature of 296℃. For P(3,4HB), the same washing time (45 s), but a higher concentration (1 mM) was required to reach the highest degradation temperature of 295℃.

When comparing unprocessed and processed samples, there was no significant difference in almost all results from TGA and DSC which means that the samples were not affected when processed in the rheometer. The results from the SEC measurement were very hard to evaluate since the signal was very unstable, so the calculations made in the computer was not accurate. The molecular weight results were very contradicting so no reliable conclusion could be made.

To be able to compare citric acid with one other acid, three different concentrations of formic acid was used to wash the biopolymer powder and further analyzed with TGA. The results were similar to the results from the citric acid wash, so further investigations were only made with citric acid.

To see how citric acid would affect the degradation temperature if not washed away, the acid was mixed into the biopolymer powder and analyzed with TGA. The result for P(3HB) showed a decrease in degradation temperature with 27℃. For P(3,4HB), there was no significant difference between the as-received powder and the as-received powder with acid. This may depend on the fact that no acid was present in the selected sample.

For curiosity, washing with deionized water was performed to see how this would affect the degradation temperature. This was evaluated with TGA and the results showed that, for P(3HB), the degradation temperature almost reached the same degradation temperature as when washed with citric acid. This result contradicts the fact that an acid is needed to stabilize the homopolymer. Unlike P(3HB), P(3,4HB) cannot be washed with deionized water and reach the same degradation temperature as for the acid wash.

In this project, error sources could be found mainly in the results from the SEC and DSC analysis. The SEC results were affected by the human factor when calculating the molecular weights, and by which plastic peace of the processed sample that was selected for the analysis.
A major error source for the DSC was the fact that P(3,4HB) was heated to a temperature to high above its melting temperature, which affected the sample in a way that no crystallization occurred during cooling.

5.1 Future work

If more time had been available, a comparison between unprocessed, as-received powder with unprocessed, washed powder in the SEC would have been done to see if the molecular weight was changed.
Even shorter washing times would be interesting to investigate, and to find out the reason why the biopolymers became more stable when washed with acid and deionized water.
It would also be interesting to see how the samples look like at different temperatures during DSC measurement.
Also, it would have been interesting to investigate how the temperature of the washing solution would affect the results.

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7 Appendix

7.1 Appendix 1

A solution concentration of 5 mg sample/ 1 ml chloroform was desired for the SEC analysis. A volume of around 5 ml chloroform was used to dissolve around 30 mg of sample. The sample weight and the corresponding chloroform amount can be seen in Table 41 below.

Table 41. The weights and amounts of chloroform for the samples used.

Sample	Weight (mg)	Amount of chloroform (ml)
P(3HB) As received, Before	25,1	5,0
P(3HB) As received, After	31,8	6,4
P(3HB) 0.05 mM, 30 min	32,7	6,5
P(3HB) 1000 mM, 30 min	28,6	5,7
P(3HB) 0.05 mM, 45 s	35	7,0
P(3HB) 1 mM, 45 s	26,2	5,2
P(3,4HB) As received, Before (1)	25,1	5,1
P(3,4HB) As received, Before (2)	36.4	5.1
P(3,4HB) As received, After	Unable to dissolve	Unable to dissolve
P(3,4HB) 0.05 mM, 30 min	25,1	5,0
P(3,4HB) 0.25, 30 min	30,1	6,0
P(3,4HB) 1 mM, 30 min	30,1	6,0
P(3,4HB) 0.05 mM, 45 s	34,4	6,9
P(3,4HB) 1 mM, 45 s (1)	21,2	4,2
P(3,4HB) 1 mM, 45 s (2)	21,2	4,2
P(3,4HB) 1 mM, 45 s, New piece	29.4	5.9

7.2 Appendix 2

To calculate the amount of acid powder/solution to give a concentration of 1 mM, the following equations were used:

$$n = c \cdot V$$

$$m = M \cdot n$$

$$c = n \cdot \rho$$

$$c_1 \cdot V_1 = c_2 \cdot V_2$$

$$n: Amount\ of\ substance\ (mole)$$

$$c: Concentration\ (mole/l)$$

$$V: Volume\ (l)$$

$$m: Mass\ (g)$$

$$M: Molecular\ weight\ (g/mole)$$

$$n: Amount\ of\ substance\ (mole/kg)$$

$$\rho: Density\ (kg/l)$$

Calculations:

Citric acid (powder)

$$n = 10^{-3} \cdot 0.45 = 4.5 \cdot 10^{-4}\ mole$$

$$m = 210.1 \cdot 4.5 \cdot 10^{-4} = 0.095\ g$$

Hydrochloric acid (solution): 37%

$$1\ kg \rightarrow 370\ g$$

$$n = \frac{370}{36.46} = 10.14\ mol/kg$$

$$c_1 = 10.14 \cdot 1.20 = 12.16\ mol/l$$

$$V_1 = \frac{4.5 \cdot 10^{-4}}{12.16} = 3.70 \cdot 10^{-5}\ l = 37 \mu l$$

Acetic acid (solution): 100%

$$1\ kg \rightarrow 1000\ g$$

$$n = \frac{1000}{60.05} = 16.65\ mol/kg$$

$$c_1 = 16.65 \cdot 1.05 = 17.49\ mol/l$$

$$V_1 = \frac{4.5 \cdot 10^{-4}}{17.49} = 2.57 \cdot 10^{-5}\ l = 25.7\ \mu l$$

Formic acid (solution): 95%

$$1\ kg \rightarrow 950\ g$$

$$n = \frac{950}{46.03} = 20.64\ mol/kg$$

$$c_1 = 20.64 \cdot 1.22 = 25.18\ mol/l$$

$$c_2 = \frac{25.18 \cdot 10^{-4}}{5 \cdot 10^{3}} = 0.50\ mol/l$$

$$V_2 = \frac{4.5 \cdot 10^{-4}}{0.50} = 8.94 \cdot 10^{-4}\ l = 894\ \mu l$$

7.3 Appendix 3

To calculate the amount of acid powder/solution to give a concentration of 0.05, 0.15, 0.25, 0.75, 1, 10, 100 and 1000 mM, the following equations were used:

$$n = c \cdot V$$

$$m = M \cdot n$$

$$n: Amount\ of\ substance\ (mole)$$

$$c: Concentration\ (mole/l)$$

$$V: Volume\ (l)$$

$$m: Mass\ (g)$$

$$M: Molecular\ weight\ (g/mole)$$

$$n: Amount\ of\ substance\ (mole/kg)$$

Calculations:

$$c \cdot 0.45 = n\ (mole)$$

$$210.1 \cdot n = m\ (g)$$

$$c_1 = 0.05\ mM, \quad n_1 = 2.3 \cdot 10^{-5}\ mole, \quad m_1 = 0.0047\ g$$

$$c_2 = 0.15\ mM, \quad n_2 = 6.8 \cdot 10^{-5}\ mole, \quad m_2 = 0.0142\ g$$

$$c_3 = 0.25\ mM, \quad n_3 = 1.1 \cdot 10^{-4}\ mole, \quad m_3 = 0.024\ g$$

$$c_4 = 0.75\ mM, \quad n_4 = 3.4 \cdot 10^{-4}\ mole, \quad m_4 = 0.071\ g$$

$$c_5 = 1\ mM, \quad n_5 = 4.5 \cdot 10^{-4}\ mole, \quad m_5 = 0.095\ g$$

$$c_6 = 10\ mM, \quad n_6 = 0.005\ mole, \quad m_6 = 0.95\ g$$

$$c_7 = 100\ mM, \quad n_7 = 0.045\ mole, \quad m_7 = 9.5\ g$$

$$c_8 = 1000\ mM, \quad n_8 = 0.45\ mole, \quad m_8 = 95\ g$$