

**MODIFICATION OF CORN STARCH BY
OXIDATION AND ACETYLATION**

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**by
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ABSTRACT

MODIFICATION OF CORN STARCH BY OXIDATION AND ACETYLATION

More sustainable and eco-efficiency industries intend to use bio-based raw materials instead of petroleum-based raw materials. Starch is one of the most useful, easily accessible, and cheap bio-based raw materials in the industry. This study aimed to produce modified starch that had proper solution viscosity for coating and low gelling enthalpy to reduce production energy for the adhesive industry. Corn starch was modified by oxidation with sodium hypochlorite (NaOCl), acetylation with acetic anhydride (AA), and a combination of the two (dual modification). The effect of different amounts of oxidants (6.0, 12.0, and 24.0 g Cl/ kg starch) and acetylation agents (7.50, 15.0, 30.0 g AA) on the properties of corn starch was examined at room temperature. The structural characteristics of starch granules were assessed using FTIR, ¹H-NMR, DSC, solution viscosity, light transmittance, and SEM microphotographs. The effectiveness values that were identified as carbonyl and carboxyl percentage and degree of substitution (DS) in modified starches were determined. The results showed that increasing oxidant concentration increased the carbonyl and carboxyl percentage to 0.0061 % and 1.33 % but acetyl groups were independent from increasing acetic anhydride concentration. Oxidation had a superior reduction effect on starch solution viscosity with 42% reduction when compared to 22% reduction with acetylation. Both processes reduced the gelling enthalpy, but double modification was more effective. Acetylation had a greater effect on light transmittance. Surface morphology of starch was only affected by active chlorine content at 24.0 g Cl/ kg starch, but all acetic anhydride amounts caused the changes.

ÖZET

MISIR NİŞASTASININ OKSİDASYON VE ASETİLASYON İLE MODİFİKASYONU

Değişen dünya koşullarında yenilenemeyen kaynakların her geçen gün daha tükenme tehlikesiyle karşı karşıya kalması sonucunda endüstriyel hammaddelerde petrol bazlı hammaddeler yerine doğal kaynaklardan elde edilen hammaddeler kullanmaya yönelinmiştir. Bu amaç doğrultusunda yaygın bulunan nişasta gibi biyobazlı doğal hammaddelerden petrol bazlı olanlarla aynı performansın alınması için farklı modifikasyon yöntemleriyle modifiye edilmiş ve performansları arttırılmıştır. Bu çalışmada, tutkal endüstrisinde kullanılmak üzere kaplama viskozitesine uygun, düşük jelleşme sıcaklığına ve entalpisine sahip ikili modifikasyona uğramış nişasta eldesi gerçekleştirilmiş ve modifikasyon işlemleri sırasında farklı miktarlarda kullanılacak oksidasyon ajanı olan sodyum hipoklorit (6.0, 12.0, and 24.0 g Cl/ kg nişasta) ve asetilasyon ajanı olan asetik anhidritin (AA) (7.50, 15.0, 30.0 g AA) modifikasyon verimine, modifiye edilen nişastanın fiziksel ve kimyasal özelliklerine, nişastanın termal yapısına etkisine ve sulu çözeltisindeki değişimin etkisine olan katkısı incelenmiştir. Bu amaç doğrultusunda modifiye edilen nişastaların asit baz Titrasyon yöntemiyle karbonil, karboksil ve yer değiştirme dereceleri ölçülmüş, FTIR, ¹H-NMR, DSC, SEM cihazlarıyla karakteristik analizleri gerçekleştirilmiş sulu çözeltisinin viskozitesi ve ışık geçirgenliği ölçülmüştür. Elde edilen sonuçlara göre, karbonil ve karboksil derecesinin oksidant miktarı ile arttığı ve %0.0061 ve %1.33 değerleri elde edilirken yer değiştirme derecesinin asetilasyon ajanından bağımsız olduğu görülmüştür. Oksidasyon işleminin %42 viskozite düşüşüyle asetilasyon işleminin ise ışık geçirgenliğinin arttırılmasında baskın olduğu iki prosesin de jelleşme sıcaklığı ve entalpisinin düşürülmesinde etkin olduğu fakat ikili modifikasyonun daha baskın olduğu gözlemlenmiştir. Oksidasyon işleminde 24.0 g Cl/ kg nişasta oksidant miktarına kadar morfolojik olarak değişiklik gözlemlenmediği ve okside nişasta asetatlarında ise her miktarda yüzeyde bozulma olduğu gözlemlenmiştir.

TABLE OF CONTENTS

LIST OF FIGURES	vii
LIST OF TABLES	ix
CHAPTER 1. INTRODUCTION	1
CHAPTER 2. BACKGROUND INFORMATION	4
2.1 Starch	4
2.2 Starch Modification Methods	12
2.2.1 Physically Modification of Starch.....	13
2.2.1.1 Thermal Treatments.....	13
2.2.1.1.1 Heat-Moisture Treatments.....	13
2.2.1.1.2 Pregelatinization.....	14
2.2.1.1.3 Heating of Dry Starch.....	14
2.2.1.1.4 Preparation of Granular Cold-Water-Swelling Starch .	14
2.2.1.1.5 Annealing	15
2.2.1.2 Nonthermal Treatments	15
2.2.1.2.1 Milling.....	15
2.2.1.2.2 High-Pressure Treatment.....	16
2.2.1.2.3 Ultrasonication	16
2.2.1.2.4 Pulsed Electric Field.....	16
2.2.2 Enzymatically Modification of Starch	16
2.2.3 Chemical Modification of Starch	17
2.2.3.1 Substitution Reactions	17
2.2.3.1.1 Esterification Reaction	19
2.2.3.1.2 Etherification Reaction.....	23
2.2.3.2 Depolymerization Reactions	24
2.2.3.2.1 Acid Hydrolysis.....	25
2.2.3.2.2 Dextrinization.....	25
2.2.3.2.3 Oxidation	25
2.2.3.3 Crosslinking Reactions.....	28
CHAPTER 3. LITERATURE SURVEY.....	30
CHAPTER 4. MATERIAL AND METHODS.....	43

4.1 Materials	43
4.2 Synthesis of Oxidized Starch Acetate.....	43
4.3 Characterization of Oxidized Starch Acetate.....	45
4.3.1 Effectiveness of Oxidation and Acetylation Processes.....	45
4.3.2 Thermodynamic Characteristics of Gelatinization by DSC.....	46
4.3.3 Granules Morphology Analysis	46
4.3.4 FTIR	47
4.3.5 UV-VIS Spectrometry.....	47
4.3.6 ¹ H-NMR Spectra	47
4.3.7 Solution Viscosity of Starch	48
CHAPTER 5. RESULT AND DISCUSSION	49
5.1 Effect of Oxidant Concentration	49
5.2 Effect of Acetylation Agent Concentration	58
CHAPTER 6. CONCLUSION	66
REFERENCES	68

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 2.1. Amylose (a) and amylopectin (b) structures in starch.....	5
Figure 2.2. Glucose unit in amylose and amylopectin unit.	6
Figure 2.3. Usage areas of modified starch.	10
Figure 2.4. General modification methods of starch.	12
Figure 2.5. Reaction mechanism and agents of etherification and esterification reactions.	18
Figure 2.6. Reaction mechanism of acetylation process.....	21
Figure 2.7. The reaction mechanism of starch succinate.	22
Figure 2.8. The reaction mechanism of etherification.	24
Figure 2.9. Common oxidation reactions.....	26
Figure 2.10. Oxidation with sodium hypochlorite.....	27
Figure 3.1. Morphologies of starch in PLM and electron SEM. Native starch (A, a), starch oxidized with 2% active chlorine (B, b), and with 4% active chlorine (C, c), m: polarization cross weakened, p: pores, q: crack.	31
Figure 3.2. Consumption of sodium hypochlorite during oxidation reaction with different pH values.....	32
Figure 3.3. FTIR Spectrum of native and modified starch	33
Figure 3.4. SEM images of native (1) and acetylated (2) corn (1E-2E) and potato starch (1A-1B-1C-1D-2A-2B-2C-2D).....	36
Figure 3.5. Effect of esterification reaction condition (a) time, (b) temperature, (c) amount of acetic anhydride, (d) water, (e) pH on the degree of substitution (DS) and reaction efficiency (RE) ((cont. on next page)	38
Figure 3.6. FTIR spectroscopy of native (a) and acetylated corn starch (b).....	40
Figure 4.1. Experimental Configuration of (a) oxidation and (b) acetylation processes. 44	
Figure 5.1. FTIR spectra of natural, oxidized, and acetylated oxidized corn starches for different oxidant levels.....	51
Figure 5.2. DSC and DDSC curve of unmodified corn starch	52

<u>Figure</u>	<u>Page</u>
Figure 5.3.Scanning electron micrographs of natural and modified starches with different oxidant ratios. Native starch (a), Oxidized I (b), Oxidized II (c), Oxidized III (d) Acetylated oxidized I (e), Acetylated oxidized II (f), Acetylated oxidized III (g)	55
Figure 5.4.Scanning electron micrographs of oxidized I (a) and acetylated oxidized I (b).....	56
Figure 5.5.H-NMR spectra of (a) natural corn starch, (b) Oxidized II, and Acetylated oxidized II.....	58
Figure 5.6.FTIR spectra of natural, oxidized, acetylated, and acetylated oxidized starches	60
Figure 5.7.DSC and DDSC curve of Acetylated Oxidized II.....	61
Figure 5.8.Scanning electron micrographs of natural and modified starches with different esterification agent ratios. Native starch (a), Oxidized II (b), Acetylated oxidized VI (c), Acetylated oxidized II (d), and Acetylated oxidized V (e).....	63
Figure 5.9. ¹ H-NMR spectra of acetylated oxidized corn starches with different esterification agent concentration	65

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 2.1.Gelling characteristics of different starches .	7
Table 2.2.Size, shape, and amylose content of some starch granules.....	8
Table 2.3.Starch composition	9
Table 2.4.General esterification agents.....	20
Table 3.1.Effect of pH on oxidation process	35
Table 3.2.Viscosity changes of potato starch with different oxidant concentration.....	36
Table 3.3.Carboxyl, carbonyl content, and acetyl groups in modified starch	41
Table 3.4.Thermodynamic characteristics of native corn starch and modified	42
Table 5.1.Effect of oxidation concentration on corn starch oxidation and acetylation ..	50
Table 5.2.Thermal properties of natural and oxidized and acetylated starch for different oxidant concentration	52
Table 5.3.Effect of oxidant concentration on light transmittance properties of oxidized and acetylated corn starch	54
Table 5.4.Solution viscosity of natural and modified starches	56
Table 5.5.Acetylation of natural and oxidized II starches with different acetic anhydride amounts	59
Table 5.6.Thermal properties of modified starch with different acetic anhydrite amount	61
Table 5.7.Light transmittance of modified starch with different acetylation agent amount	62
Table 5.8.Solution viscosity of natural and modified corn starches	64

CHAPTER 1

INTRODUCTION

Considering ideals like dematerialization, factor 4, factor 10, eco-efficiency, and green innovation industrial applications, products, and raw materials must become more sustainable and/or 'green' from present methods (Borza 2014). Also, besides this trend, changing world, and industrial requirements necessitate this change. With this alteration petroleum-based, raw materials have been started to replace with bio-based raw materials. Biopolymers have been present for quite a great many years and have always been an important component of sustainability, first and mainly as food, but also as housing, clothing, and a source of energy. The evolving technologies associated with papermaking, fabric and wood processing, the use of vegetable oils, starch, and gelatin, glue manufacture, and so on are clear examples of the increasing complexity with which people have made great use of all-natural polymers throughout millennia (De Jong 2012).

Among all these bio-sources, starch is one of the low-cost, easily available, and useful raw materials used in a variety of sectors, including the chemical, food, glue, and paper industries (Acquavia 2021). Natural starch is made up of two major polymeric parts amylose and amylopectin. Amylose is a linear large molecule; amylopectin is a branched chain. According to these polymers' ratios, and properties of starch can be changed. The degree of polymerization (DP) of amylose and amylopectin, as well as the availability of other constituents in the granule such as lipids, proteins, and inorganic materials, are other essential variables determining these features. Also, origin, type, granule shape, and size are the important parameter of starch for its quality. Corn starch is one of the most accessible, cheapest, good-quality starch types. Its granule structure and size are suitable for its modification (Maniglia 2021)

Although native starches are high-quality raw materials, they have a lot of drawbacks that limit their use in industrial applications. It is not always possible to create the required functional qualities (grain stability, solubility in cold water, thickening strength after cooking) with native starch. These limits can be overcome by creating modified starches with improved functional properties with physical modifications (thermal, and non-thermal treatment), chemical modifications (degradation, substitution, and crosslinking), and enzymatic modification (degradation) or by a combination of these methods. When all these techniques are considered, chemical and physical methods are frequently favored in the industry due to their ease of application. Enzymatic modification is quite expensive for the industry. Physical modifications are more environmentally friendly than others since they do not emit harmful pollutants. Among all these methods, oxidation and esterification methods are chemical modification methods that are widely used methods in the industry especially food, paper, and glue industries.

In oxidation methods, hydroxyl groups (-OH) in anhydroglucose units (AGU) of starch are replaced with the carbonyl (-C(=O)-) and carboxyl (-COOH) groups that come from the oxidation agents and also glycoside bonds are broken, and starch molecules are decomposed. Thanks to this modification, starch molecules become smaller. These changes prepare and make easier it for second modification (Xie 2005). Different oxidants have been used to oxidize starch, including periodate, hypochlorite, hydrogen peroxide, permanganate, persulfate dichromate, and chlorite. Hypochlorite is new, and widely used oxidation agent in industries because of its cost and accessibility. The efficiency of these modification types (carbonyl and carboxyl percentage) is measured by the acid-base titration method. For this modification, types are affected by reaction time, temperature, pH, solution medium solid content, and catalyst type (Masina 2017).

Esterification is the process that which hydroxyl groups of starch are substituted with ester groups (RCOOR') that comes from the esterification agents. Mostly acid and its anhydride are used. Common esterification agents are acetic anhydride, succinic anhydride, vinyl acetate, sodium tripolyphosphate, and 1-octenyl succinic anhydride for this process. Acetic anhydride (acetylation), and succinic anhydride (succinate) which is a widely used method in the food, adhesive, and textile sectors. By reacting starch

with acetic anhydride, starch acetates are created. Acetic anhydride is a widely preferred chemical for this process in the industry. The number of groups integrated into the compound is controlled by the pH, catalyst presence, reactant concentration, and reaction time, and this efficiency is the measured degree of substitution (DS) value by acid-base titration. In some situations, one modification cannot run short of intended properties. In this case, double modification can be used to increase specifications. The first modification makes the second modification easy. It might break, open the bonds or change the charge of groups of starch (Masina 2017).

The objective of this thesis was to investigate the effect of modification of corn starch by the oxidation with sodium hypochlorite and acetylation with acetic anhydride and using their combination (dual modification) to reduce solution viscosity and gelling temperature of starch for the adhesive industry. Modification degrees that named carbonyl and carboxyl percentage in oxidation and degree of substitution (DS) in acetylation are significant for intended properties. For this purpose, sodium hypochlorite (and acetic anhydride will be used in different dosages to reduce gel viscosity and gelling temperature and increase stability for adhesive industry. The modified starch will be characterized by FTIR spectroscopy, DSC, SEM, UV-VIS-NIR Spectrophotometry, and $^1\text{H-NMR}$. Their carbonyl, carboxyl contents, and degree of substitution (DS) will be determined by acid-base titration methods.

CHAPTER 2

BACKGROUND INFORMATION

2.1 Starch

In-plant kingdom, starch is the primary carbohydrate resource. In opposition to cellulose, which is found in dietary fibers, starch is metabolized by humans and is one of the primary energy sources required to support life. Bread, rice, and potatoes are all instances of how important carbohydrate is in our culture. Starch is also used for millennia in a variety of non-food uses, such as glue for wood and paper and gum for the textile industry. Starch, along with wood, and natural fibers has been a preferred material from the dawn of human technology. Starch is unquestionably one of the most materials with possible applications in polymer technology. On the one side, it may be transformed into chemicals like acetone, ethanol, and organic acids, which are used in the manufacture of synthetic polymers; on the other hand, it can even be fermented and used to generate biopolymers, or it can be hydrolyzed and utilized as a monomer or oligomer. Furthermore, it may be grafted with a variety of chemicals to create novel polymeric materials that can be utilized as is or as fillers for other polymers (Belgacem 2011).

Many plants are used extensively to produce starch. Sources of starch include cereal grain seeds (wheat, rice, sorghum, and maize), roots (sweet potato, tapioca, and arrowroot), tubers (potato), and sago palm pith. Each starch is labeled with its plant source, such as potato starch, maize starch, wheat starch, and so on. (Swinkels 1998) The plant of choice is determined mostly by regional and climatic variables, as well as the necessary functional qualities of the matching starch. Whatever the temperature and agricultural circumstances, it can discover a very productive plant to generate starch: potato in cold environments, cassava (manioc or tapioca) and banana in subtropical settings, maize in temperate and tropical zones, and rice in flood-prone areas.

Starch may be present in many different areas of a plant, including the endosperm, root, leaf, and fruit pulp. It is formed in the form of semi-crystalline granules that like spherulites with alternating crystalline and amorphous (or semi-crystalline) lamellae and is insoluble in cold water (Pietrzyk 2018). Natural starch is made up of two major polymeric parts: amylose and amylopectin. These natural polymers' monomer units are crystalline form molecules connected by (1-4)- α and (1-6)- α links. Amylose is a linear large molecule that is mostly (almost 99%) - (1-4)- α -D-glucopyranosyl. Amylopectin is indeed a branched-chain, high molecular weight macromolecule consisting mainly (almost 95%) α -(1-4)-D-glucopyranose units with - α (1-6)-linkages at roughly 20-unit intervals (Pietrzyk 2018). Figure 2.1 shows the structure of these two main polymers.

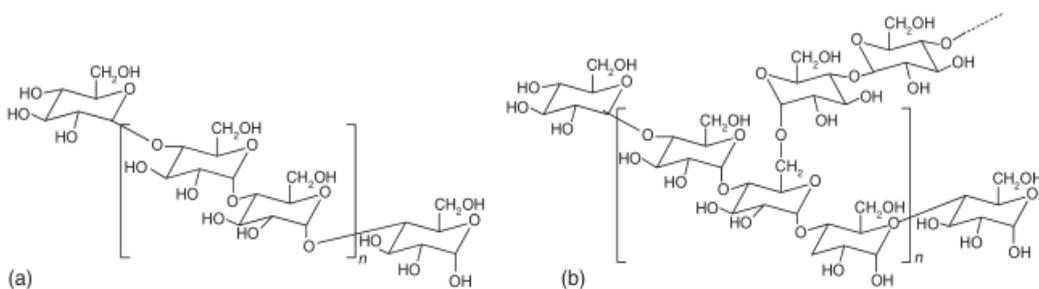


Figure 2.1. Amylose (a) and amylopectin (b) structures in starch

(Source: Paul 2016)

Several mutations of corn, rice, and sugarcane are developed for their unique starch properties. The waxy grains (waxy sorghum, waxy maize, and waxy rice) have starch but no amylose component (100% amylopectin). Plant breeders have created corn types containing 50-80% amylose in the starch molecules (Swinkels 1998).

Amylose and amylopectin polymers are made of glucose units as shown in Figure 2.2. The starch molecules' glucose units have a primary hydroxyl group on C6 and a secondary hydroxyl group on C2 and C3. Starch granules include many hydroxyl groups, which provide its hydrophilic characteristics and cause it to disperse when heated with water. However, these hydroxyl groups usually attract one another,

establishing hydrogen bonds between nearby starch granules and preventing starch granules from dissolving in cold water below their gelling temperature.

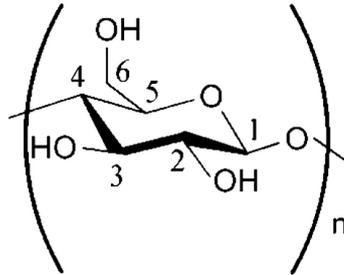


Figure 2.2. Glucose unit in amylose and amylopectin unit

(Source: Li 2019)

All these properties affect the solubility, swelling, and gelatinization of starch. These parameters are quite important for starch's processability. Gelatinization describes the swelling of starch irreversibly. The first swelling occurs in the amorphous portions of the particle, breaking and wetting the weak connection between starch granules. When aqueous starch solution temperature rises, more hydrations happen in the amorphous areas, and hydrogen bonds in the crystalline regions start to break down. The granules continue to grow into a big, reticulated network, kept together by persistent micelles that have not been disturbed. The crystalline sections are disrupted when there is a lot of swelling. Shorter micelles dissolve during the earliest phases of gelatinization, but longer micelles remain at higher temperatures. As the mixture is heated and agitated, the swollen starch particles break and fall, resulting in a viscous colloidal solution of swelling granule fragments, hydrated starch aggregates, and dissolved molecules. When the starch solution temperature is raised, the first granules begin to gelatinize at a specific degree. At higher temperatures, other granules in the same mixture, generally smaller in size than the first, begin to gelatinize. This means that the gelatinization of a starch solution cannot be characterized as occurring at a specific temperature, but rather as occurring within a specific temperature range. (Swinkels 1998). Gelatinization characteristic specialties of natural starch are demonstrated in Table 2.1.

Table 2.1. Gelling characteristics of different starches (Swinkels 1998).

Starch	Pasting Temp. (°C)	Peak Viscosity %5 starch conc. (cP)	Swelling Power at 95°C	Solubility Power at 95°C (%)
Potato	60-65	1000-5000	1153	82
Corn(Maize)	75-80	300-1000	24	25
Wheat	80-85	200-500	21	41
Tapioca	60-65	500-1500	71	48
Waxy Corn	65-70	600-1000	64	23

Tapioca and potato starch have a lower pasting temperature than maize and wheat starch, as demonstrated in Table 2.1. When the starch solution temperature rises, the granules swell, and the viscosity of the starch solution increase. This process is performed until "peak viscosity" is achieved. The peak viscosity is the greatest viscosity that a user may experience while making a starch paste. Toot and tuber starches have a greater peak viscosity and a faster rise in viscosity during boiling than cereal starches. The peak viscosity of potato starch is the greatest. Because the particles in corn starch are only mildly swollen, the maximal viscosity is comparatively modest. When a starch aqueous solution is heated just above gelling temperature, the particles expand gradually. The swelling power may be evaluated at 5°C intervals over the whole pasting temperature range of about 50 - 95 °C. When heated in water, granules of tapioca, potato, and waxy corn starch disintegrate more quickly than grains of corn and wheat starch, allowing them to attain the homogenous state required for many applications more quickly. (Swinkels 1998).

When a dilute starch solution is left to stand for an extended period, it gradually gets cloudy and finally forms an insoluble precipitate. When a highly concentrated starch solution is cooled down, it quickly solidifies into an elastic gel. Both are retrogradation mechanisms in which the starch material transitions from a dissociated and dissolved state to an attached state. The crystalline micelles in starch granules are mostly composed of amylopectin molecules. In contrast, retrogradated, crystalline starch is mostly made up of amylose molecules. In the retrogradation stage, opacity and

turbidity develop, viscosity increases on heated pastes, insoluble starch particle precipitation is shown, and the formation of insoluble "skins" occurs, the formation of gels occurs. Retrogradation is a complicated process that is affected by several elements such as starch type, starch content, temperature, cooking process, pH, cooling process, storage period, and the presence of other chemicals. Reduced temperatures and high starch concentrations are typically favorable for the retrogradation of starch dispersions. The rate of retrogradation is the highest at pH 5-7 and slows as pH increases and decreases. Retrogradation does not happen at pH 10 and occurs slowly below pH 2 (Matignon 2017)

The shape of starch granules, and also the structure of its primary polymeric elements, have been the subject of extensive research study which is currently underway due to the complexities of the major difficulties. The form of the starch molecules differs not only by source plant but also by portion with the same plant. The degree of polymerization (DP) of amylose and amylopectin, as well as the availability of other constituents in the granule such as lipids, proteins, and inorganic materials, are other essential variables determining starch features. The functionality of starches is also affected by these starch's properties. Shape, particle size, amylose, and amylopectin percentage are important parameters for it. Table 2.2 and Table 2.3 show specialties that influence the starch functionality of different starches.

Table 2.2. Size, shape, and amylose content of some starch granules (Thakur 2019)

Source	Diameter (μm)	Amylose Content (wt %)	Shape
Corn(Maize)	5-25	25	Polyhedric
Waxy Maize	5-25	~0	Polyhedric
High Amylose	5-35	55-85	Varied smooth spherical of elongated
Cassava	5-35	16	Semi-spherical
Potato	15-100	20	Ellipsoidal
Wheat	20-22	30	Lenticular, polyhedric

Table 2.3. Starch composition (Swinkels,1998)

Source	Starch (%)	Moisture (%)	Protein (%)	Lipids (%)	Fiber (%)	Starch on dry substance (%)
Potato	17	78	2	0.1	1	77
Corn	60	16	9	4	2	71
Wheat	64	14	13	2	3	74
Tapioca	26	66	1	0.3	1	77
Waxy Maize	57	20	11	5	2	71

As shown in the Tables, starch types have different properties according to their origin and range. The roots and tubers differ from grains in that they also have a significantly higher water content but a lower amount of fat. All these properties affect the starch applicable and its product. The starch percentage, evaluated on a dry basis, is around 70-80 percent for all-natural resources. Corn (Maize) and potato starch are widespread starch types in the industry with their extensity, accessibility, and price. The granules of potato starch are circular, with a smooth surface. The particles of potato starch are the biggest of any industrial starch. Potato starch manufacturing accounts for around 3% of the global potato crop. Potato starch is manufactured in around 2 million tons across the world, with the Netherlands producing 500.000 tons. The granules of corn starch are medium in size and irregular angled shape. Only around 6% of the world's corn harvest can be used for starch production each year. The global output of corn starch is around 16 million tons, with the United States producing approximately 8 million tons. Maize starch contributes to more than 75% of total global starch output. (Swinkels 1998). As shown in Table 2 they have almost the same amylose content, but the particle size of potato starch is higher than corn starch, because of this specialty potato starch might need to reduce its particle size to increase its processability. It can be a drawback for the industry since it increases the cost and time of the process (Singh 2004)

Although natural starch is a good raw material, it in its original condition has limited industrial trial uses because of its unstable viscosity, poor shear force tolerance,

and thermal degradation, along with rapid retrogradation and gelling, but these drawbacks can be solved. To improve these drawbacks, it can be modified physically (thermal, and non-thermal treatment), chemically (degradation, substitution, crosslinking), and enzymatically (degradation) or a combination of these modification methods. With these modifications, starch becomes more usable (modify cooking characteristics, decrease retrogradation, decrease gelling tendencies of pastes, increase freeze-thaw stability of pastes, decrease paste and/or gel syneresis, improve paste and/or gel clarity and sheen, improve paste and/or gel texture, improve film formation, improve adhesion, add hydrophobic groups) (BeMiller 2019). These modified starches might be used in many industries easily. Figure 2.3 demonstrates the different usage areas of modified starch.

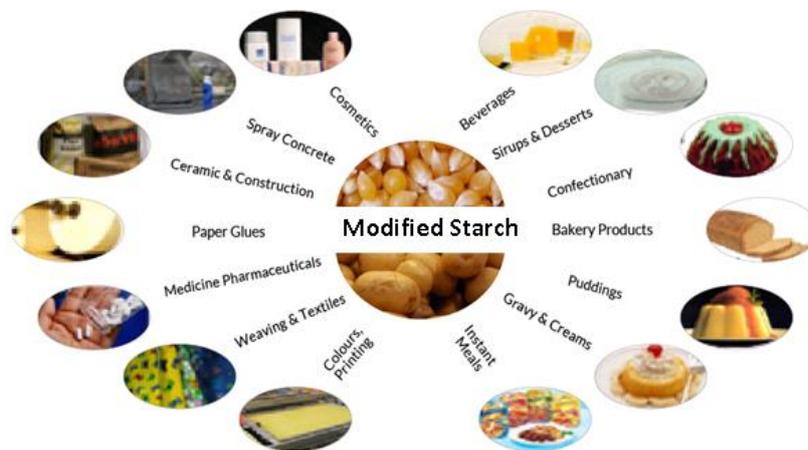


Figure 2.3. Usage areas of modified starch

(Source Starch Retrieved EICL, 2015)

As seen in the figure after physical modification, chemical modification, and enzymatic modification starch can be used for different industries with different purposes.

Besides the food industry, modified starches are used in many industries like paper, adhesive, paint, medicine, construction, and cosmetic industries. In the food industry, oxidized starches are employed in meals as binding agents, such as batters added to meats before frying. In salad dressings, cross-linked starches are utilized to produce strengthening with a constant viscosity at lower pH and high shear during the mixing process. Soups, sauces, newborn meals, pudding, and deep-fried dishes have all

used them. Furnace-dried cross-linked starches are utilized in food systems to give a papery texture. Acetate starches are thickeners found in baked goods, canned goods, frozen goods, and dry goods. They're also used in fruit and cream pies, tarts, salad dressings, and gravies. Frozen fruit pies and pasta sauces produced with acetate starch may be stored at cool temperatures (Abbas 2010).

The paper industry is one of the big nonfood user industries of starch. Starch is employed as an ingredient in the cellulose pulp before the formation of the paper sheet. And also, it is used in the surface sizing of paper, and the surface coating of paper. Starches are introduced to the pulp during internal sizing to improve the dry stiffness of the paper. They can also be utilized to improve filler and color stability. The starch bonds the individual cellulose pulp fibers together. The most common surface sizing reagent is starch. Because of the high viscosity of natural starch, modified starch that has low viscosity is used in this process. Paper surface sizing enhances the strength and surface qualities for printing and writing. The intended surface attributes include a better look, surface toughness, abrasion resistance, printability, resistance to fluid, erasability, and ink entry into the sheet (Zarski 2021). To achieve a white smooth surface, white granules (calcium carbonate, clay, titanium dioxide, or satin white) are added to the surface of paperboard and paper. Starch products are used as a binder to hold pigment particles together and to the paper. In this process, oxidized starches, starch ethers, starch esters oxidized starch ethers or esters, enzymes, and thermally modified starch can be used (Swinkels 1998).

For the adhesive industry, starch-based adhesives are ideal for bonding porous hydrophilic paperboard, and paper products. They can be utilized in any application where both faces are them, or if one is glass or metal and the other is paper. In the production of adhesives, essential raw ingredients such as potato, corn, tapioca, and waxy maize starches are employed. In gummed paper, dextrin is used to apply a thin layer of remoistening gum to the paper used to make postage stamps, gumming stamps, labels, and envelopes. As another product, water-remoistenable glue on gummed tapes is made from tapioca, potato, and waxy maize thin-boiling starches. Native starches are changed to provide low viscosity (via breakdown) and viscosity stability (by etherification and/or esterification) (Onusseit 1992). A huge amount of corn starch is utilized in the production of corrugated boards. The gelling starch holds a corrugated

strip of paper and a soft liner together. In addition to these, bag adhesive, and wall covering adhesive can be produced with starch. Starch is used textile industry for warp sizing, completing textile fabrics, and transporting color and reagents in printed pastes.

2.2 Starch Modification Methods

The characteristics of starch are adjusted using several approaches to fulfill today's increasingly competitive requirements. Starch modification tries to overcome one or more of the aforementioned flaws, increasing its applicability and satisfying market demand. These changes are proposed to integrate beneficial changes in the starch, allowing for predictable and regulated activity. As a result, modified starch compounds are the result of glycosidic bond cleavage (acid modification to dextrin) or the formation of new functional groups (carbonyl group formation during oxidation), or the substitution of free available hydroxyl groups (by etherification or esterification), or the bridging of molecular chains by cross-linking reactions (Tharanathan 2005). The arrangement of the inserted chemical substituents and residual free hydroxyl groups inside the anhydroglucose (AGU) residue and all along the polymer backbone can have a significant impact on the product qualities in these functionalizations. Physically, non-destructive, and degradative chemically and enzymatically alterations are among the modifications used. Figure 2.4 shows the general modification methods and their special reaction types.

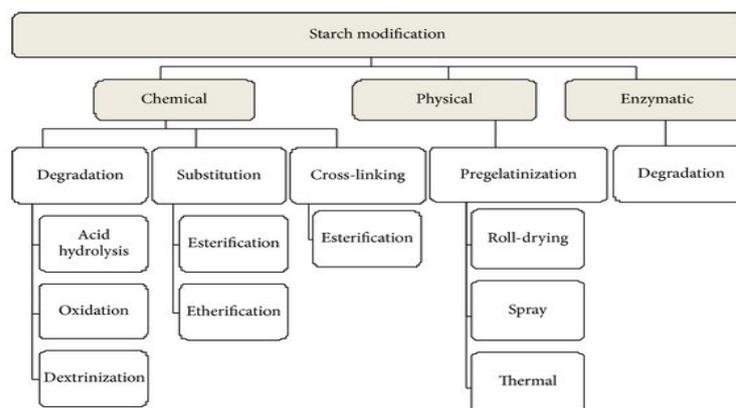


Figure 2.4. General modification methods of starch

(Source: Lewicka 2015)

As shown in the figure different modification methods can be used to modify starch to achieve the intended properties of the product.

2.2.1 Physically Modification of Starch

Physical modification of starches has recently attracted a lot of attention. Physical modifications are those that occur as a result of treatment techniques that do not modify the chemical configurations of the D-glucopyranosyl units of starch polymers but may result in some depolymerization (chain breaking). Physical modification of starch can be performed alone or in combination with chemical processes to change the granular structure and turn native starch into micro crystallite starch or cold soluble in water starch. Mechanical treatments often alter or disrupt the packing arrangements of amylopectin and amylose components inside granules. Starch's properties and functions can be altered by structural changes, although they are rarely as large or as thermally stable as those gained through chemical changes.

If physical changes can offer sufficient functions, they are much less costly and simpler than chemical changes, and they produce no contaminants (unreacted reagents, salts, or byproducts) making the system easier more desirable, and "greener" (BeMiller 2019). Thermal and non-thermal modifications are the two most common types of physical modifications. Thermal physical modifications; heat-moisture treatments, gelatinization, heating of dry starch, preparation of granular cold-water-swelling starch, annealing. Some non-thermal modifications: milling, high-pressure treatment, ultrasound, pulsed electric field.

2.2.1.1 Thermal Treatments

2.2.1.1.1 Heat-Moisture Treatments

Heat-moisture treatments (HMT) include the hydrolysis of wet starch granules. A wide range of conditions can be used depending on the kind of starch, water content (generally 20%–30%), temperature (typically 80–140°C), heating time (typically 1–24 hours), and heat source.

Common property effects that occur during HMT include increases in hot-paste viscosity and pasting temperature, as well as decreases in peak viscosity, granule swelling, breakdown (increased stability), and solubility, offering the starch cooking properties similar to those of a gently crosslinked starch. Common changes include an increase in gel stiffness, an extension of the gelling temperature range, and the creation or perfection of amylose-native lipid complexes (Ojogbo 2020)

2.2.1.1.2 Pregelatinization

Pregelatinization of starches (sometimes known as "instant starches") are commercially available pre-cooked starches. These are starches that have been cooked and dried in a way that prevents or limits retrogradation. Although pregelatinized starches hydrate quickly and are defined as cold dissolved in water, most of them contribute viscosity to aqueous dispersions when heated (Xie, 2005). Solvent-based processing, spray cooking, drum drying, and extrusion could all be used to make pregelatinized starch. Pastry fillings, puddings, sauces, and infant meals all use pregelatinized starch as a thickening agent. To change the texture of soups, gravies, and sauces, starches with a pulpy texture are utilized (BeMiller 2019).

2.2.1.1.3 Heating of Dry Starch

Warming starch with a low (15 percent) moisture content at a temperature above 100°C, but below that which affects warm corruption, results in starches with acid, shear, and heat resistance profiles equivalent to those of chemically crosslinked starches with minor levels of crosslinking. Making the starch soluble and drying it to a moisture content of 1% at some point during recent warming energizes the physical properties (Tharanathan 2005).

2.2.1.1.4 Preparation of Granular Cold-Water-Swelling Starch

The terms "instant" and "granular cold-water-swelling starch" (GCWS, CWS) are overused. They're also called cold-water-soluble starches, even though pregelatinized starch products are usually more dissolvable in room-temperature water

under shear rate circumstances than they are in cold water. GCWS products comprise intaglio granules that have been gelatinized. When placed in aqueous frameworks at room temperature, amorphous particles swell dramatically (BeMiller 2019).

2.2.1.1.5 Annealing

Annealing is a hydrothermal treatment for starches that modifies their physical characteristics. It happens when starch grains with a high-water content (>40%) are kept at a temperature below even gelatinization (Xie 2005). The time it took to complete the operation ranged from minutes to days. Because of the varying configurations of possible variables (type of starch, temperature, and period), a variety of products can be generated, and it is impossible to characterize the attributes of annealed starches in a way that applies to all of them. Granules remain intact during annealing. (Sui 2018)

2.2.1.2 Nonthermal Treatments

2.2.1.2.1 Milling

Milling (also known as micronization) is most commonly utilized with flours since it results in a reduction in particle size. Milling is categorized as a nonthermal process, however because of elevated heat at the points of contact and an overall rise in temperature with time during milling, there is a thermal component involved. Milling produces a mixture of unbroken granules, damaged granules, and fragments of damaged granules, with the proportions of each dictated by the milling method and time, as well as the type of starch used. Milling can cause crystallinity reduction, a drop in gelatinization temperature, and a rise in water vapor sorption, solubility, and sensitivity to amylose activity. Milling is likely to cause chain breakage, which will lower the length of certain amylopectin units. The adhesion of a food product increases as the number of shattered amylopectin molecules increases (BeMiller 2019).

2.2.1.2.2 High-Pressure Treatment

Nonthermal food methods such as high-pressure treatments have been employed to kill microorganisms and inhibit growth enzymes. Corn starches have been subjected to a variety of high-pressure treatments, including ultrahigh-pressure (UHP) treatment and treatment obtained by running a starch solution through with a high-pressure homogenizer. The type and concentration of starch, as well as pressure, duration, and temperature are all variables. Shear, turbulence, and cavitation are all produced by high-pressure homogenizers. Granules may inflate and fracture when a starch slurry is put through an elevated homogenizer; they may also agglomerate due to surface gelatinization. As granules lose some of their crystallinity, the gelatinization temperature drops. Solubility may improve. (Ojogbo 2020)

2.2.1.2.3 Ultrasonication

When starch granules are treated with ultrasound, the properties of the granules modify. It is impossible to specify changes in starch qualities in a way that applies to all products due to differences in the natures of the starches employed and differences in treatment circumstances. Although ultrasonic energy is enough to break or even deform the crystallites of starch granules, it can have an impact on amorphous regions. Sonication reduces paste viscosity while increasing swelling power solubility, and water sorption. (Sui 2018)

2.2.1.2.4 Pulsed Electric Field

If starch granules in an aqueous solution are exposed to a pulsed electric field, they become deformed, lose crystalline nature, and the temperature of gelling drops. (BeMiller 2019).

2.2.2 Enzymatically Modification of Starch

Several enzymes are utilized in the hydrolysis of starch to change the structure of the starch and obtain the desired functionality. Enzymes break down the bonds

between α -D-glucopyranosyl residues in a (1-4) or (1-6) -step process. α -amylase, β -amylase, glucoamylase, pullulanase, and isoamylase are the most frequent enzymes for starch modification. These enzymes were discovered in the kingdoms of fungi, yeasts, bacteria, and plants (Xie 2005).

2.2.3 Chemical Modification of Starch

Starches are largely chemically changed to improve the positive characteristics of either their grainy or cooked forms, to reduce the drawbacks of either their granular or cooked states, and/or to just provide functionality that native starches cannot. Three different starch states can be modified chemically. The chemical process is carried out in a water medium until the necessary characteristics are attained in suspension, where the starch is disseminated in water. Following that, the suspension is filtered, washed, and air-dried. The paste is mixed after the starch is gelatinized with reagents in a tiny quantity of water, and the starch is air-dried when the reaction occurs. Dry starch is moistened with chemicals in a liquid medium, air dried, and then activated at an elevated temperature (i.e., 100°C) in the solid form (Xie 2005). Chemical modification of starch improves molecular consistency against high-temperature hydrolysis, mechanical shearing, and acidic, as well as acceptances electronegative, or electropositive substances, rising interaction with ions, viscosity, and lowering the retrogradation percentage of natural starch. In a chemical modification of starch, degradation, cross-linking, and substitution reactions are occurring. In esterification, and etherification reactions hydroxyl groups of starch are replaced with the groups that come from these reaction agents. Also, esterification is a crosslinking reaction. Acid-catalyzed, oxidation, and dextrinization reactions are depolymerization reactions that take place in glycosidic linkages.

2.2.3.1 Substitution Reactions

Esterification and etherification reactions are the main reactions of substitution. Starch is often stabilized by treating it with esterifying or etherifying chemicals in the presence of an alkaline catalyst. A partly substituted starch derivative's substituent groups are distributed across the three hydroxyl groups (C-2, C-3, and C-6) of the starch

molecule's AGU units. The relative reactivity of the hydroxyl groups and the type of the substitution reaction influence the distribution. Improperities in the starch chains (particularly the linear amylose molecules) prevent the creation of crystalline structures in the starch paste, causing retrogradation to be delayed (raised viscosity stability). As a result, starch esters and ethers are frequently referred to as stable starches. When additional substituents are inserted into the starch chain, retrogradation diminishes. Figure 2.5 shows the main reaction mechanism and reaction agents of these reactions.

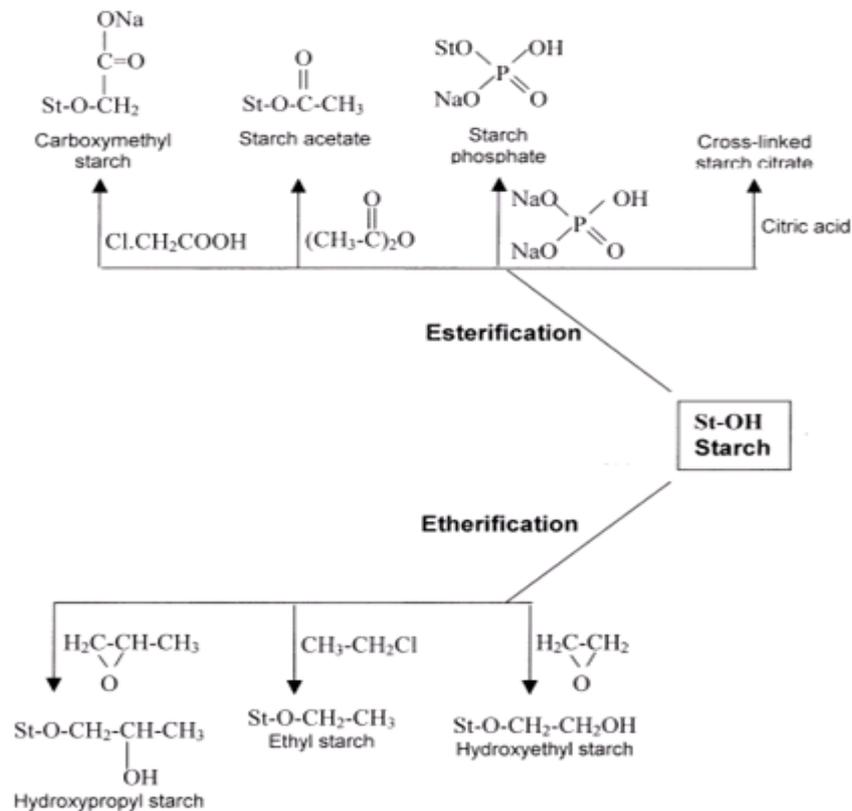


Figure 2.5. Reaction mechanism and agents of etherification and esterification reactions

(Source: Tharanathan 2005).

As observed from the figure esterification and etherification reactions are carried out by different reaction agents and end products vary according to reaction agents.

2.2.3.1.1 Esterification Reaction

A family of modified starches in which some hydroxyl groups have been substituted with ester groups is known as a starch ester. For this process, mostly acid and its anhydride are used. Common esterification agents are acetic anhydride, succinic anhydride, vinyl acetate, sodium tripolyphosphate, and 1-octenyl succinic anhydride. By reacting starch with acetic anhydride, starch acetates are created. The reactions of starch with succinic anhydride and alkenyl substituted succinic anhydride, respectively, generate starch succinate and starch alkenyl succinate. Starch phosphate is formed when starch reacts with tripolyphosphate and/or trimetaphosph. The number of groups integrated into the compound is controlled by the pH, catalyst presence, reactant concentration, and reaction time, and this efficiency (measurement of substitution) is the measured degree of substitution (DS) value. The average number of substituents per AGU unit is defined as the DS (moles substituent per mole of AGU). A starch derivative with a DS of 0.05, for instance, has 5 substituents per 100 AGU units (average value). In general, the DS of commercial starch esters and starch ethers is less than 0.2. Table 2.4 demonstrates the general esterification agents and their advantages/disadvantages and applications.

Table 2.4. General esterification agents (Ačkar 2015)

Modification Type	Advantages	Disadvantages	Application
Acetylation	Improved paste clarity	Instability during shearing at high temperatures	<i>Food industry:</i>
	Retarded retrogradation		Consistency, texture and stability improvers
	Decreased gelatinization temperature		<i>Non-food application:</i>
	Freeze-thaw stability		Tablet binders, cigarette filters, biodegradable packaging materials, coatings, adhesives etc.
Succinylation	Starch solubility in cold water	Instability during shearing at high temperatures	Thickening or stabilizing agents in soups, snacks and frozen food products
	High viscosity		
	Better thickening power		
	Improved paste clarity		
	Retarded retrogradation		
OSA * modification	Freeze-thaw stability	Decreased biodegradability	Beverage emulsion stabilizers
	Decreased gelatinization temperature		Encapsulation of flavor compounds
	Increased paste viscosity Ability to stabilize water/oil emulsions		
	Reduces glycemic response after consumption of beverages		
SCFA * modification	Nutritional role—prebiotic action	Decreased biodegradability	Potential prebiotic
	Increased water resistance		
MCFA * modification	Increased water resistance	Decreased glass transition temperature	Starch/LDPE blends
	Higher stability at high temperatures		Edible films
LCMA* modification	Increased hydrophobicity	Decreased glass transition temperature	Potential resistant starch Starch films
	Higher resistance to α -amylolysis		
Adipic acid/ acetanhydride modification	Higher paste clarity	Lower solubility	Thickening agent
	Improved paste stability		
	Higher viscosity values		
Esterification with ferulic acid chloride	Enhanced hydrophilic properties	Poor mechanical properties	Functional food ingredient
	Improved absorption of ferulic acid in intestine		

As indicated in the Table 2.4, all esterification agents have different names in the esterification process, and all of them are used for different applications some of them have disadvantages because of the substituted groups. Among all processes, the most popular and simple which is acetylation and succinate which are widely used in the food, adhesive, and textile sectors (Ačkar 2015).

Starch Acetate

Commercial production, acetylation of starch in an aqueous solution by acetic anhydride at an alkaline pH creates lower starch acetates. Starch is indirectly reacted with carboxylic anhydride under an alkaline medium. After the formation of an alkali starch complex, which reacts with the carboxyl anhydride to generate a starch ester with

the removal of the carboxylate ion and one molecule of water, a starch acetate is formed. (Reaction 1) The deacetylation (Reaction 2) of starch and the production of sodium acetate (Reaction 3) are two side chemical reactions during the acetylation process. Figure 2.6 shows the reaction mechanism of the acetylation process.

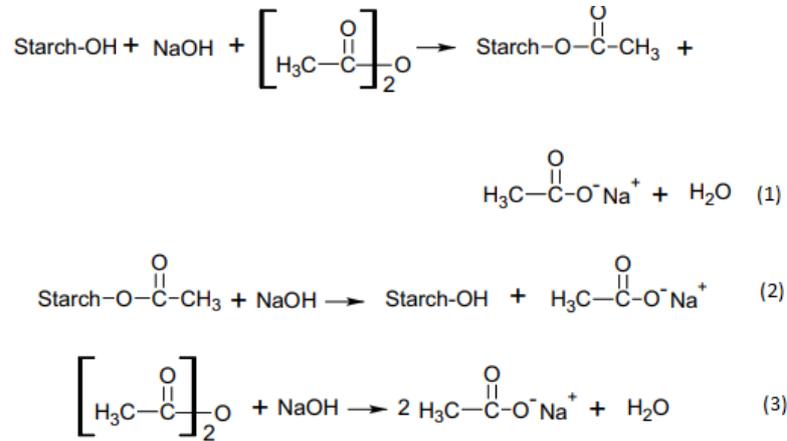


Figure 2.6. Reaction mechanism of acetylation process

(Source: Xie 2005).

In these reactions, reactant concentration, time, catalyst, time, start type, and temperature are important parameters. Increases in reactant concentration have a beneficial impact on reaction efficiency which refers to DS NaOH and H₂SO₄ are two popular catalysts used in this setting (Masina 2017). Starch is activated by the base through the formation of starch alkoxide (ST-O), which combines with acetic anhydride to create starch acetate and NaOAc. The degree of substitution is influenced by the size of starch granules and their crystallinity. As a result, acetyl groups have a harder time replacing OH groups in bigger granules and crystal regions with highly organized starch chains (Xie, 2005). The acetylation performance is influenced by reaction time. The DS rises as the reaction duration changes from 60 to 150 min, then drops as the reaction time increases to 240 min due to starch acetate hydrolysis. Increased temperature from 25 to 30 °C improves acetylating agent diffusion and starch swelling, resulting in higher substitution product yields. However, because acetylation is an exothermic reaction, increasing the temperature further might harm the development. For acetylation, water is a popular reaction media. A larger water content facilitates the esterifying agent's breakdown, diffusion, and sorption, which is beneficial to the process. Nevertheless, if

the liquid to starch proportion is too high, unintended reactions impair reaction performance

The temperature at which starch acetate gelatinizes is significantly lower than that of native starch. By raising the degree of swelling and dispersion of starch granules, acetyl-containing starch increases the stability and clarity of sols, as well as reduces retrogradation.

Starch Succinate

The interaction of starch with succinic anhydride produces starch succinate, which is a half ester. Lower DS starch succinate can be produced in the same way as starch acetate. As a result, starch succinylation with succinic anhydride is accomplished by either refluxing with pyridine or gelatinizing the starch in aqueous pyridine followed by a reaction with succinic anhydride in 100% pyridine. Pyridine has a dual purpose in the reaction: it activates starch and makes it nucleophilic, as well as reacts with succinic anhydride to generate succinyl pyridinium intermediates. When this intermediate comes into contact with starch, it produces starch succinate and pyridine (Ačkar 2015). Figure 2.7 shows the reaction mechanism of succinate.

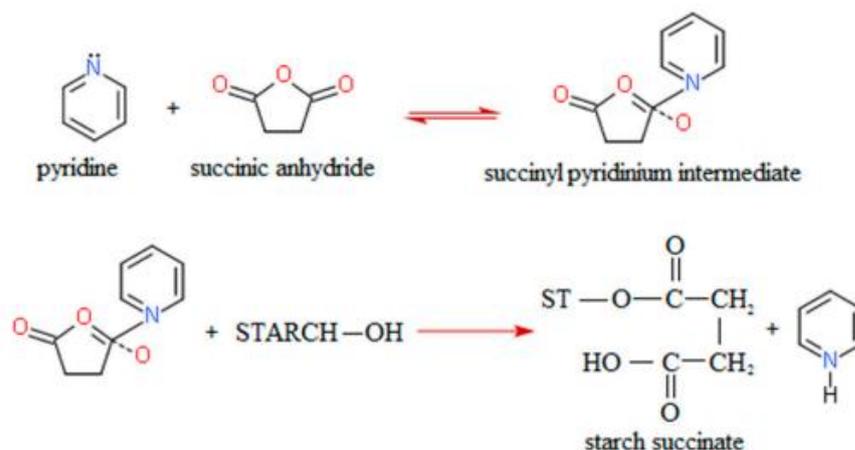


Figure 2.7. The reaction mechanism of starch succinate

(Source: Ačkar 2015)

A reasonable quantity of pyridine must be supplied to obtain an overall reaction with pyridine as the catalyst, ensuring appropriate process parameters. This is a stumbling block to this modification's industrial applicability. Pyridine, in particular, is a hazardous and costly chemical. It can be extracted from the reaction by distilling starch succinate after washing it with water, but this is an expensive method (Ačkar 2015). The unbound carboxylate group in starch succinate enhances the water retention capacity and the ability to swell in cold water. The capacity of cold water to swell improves as succinic anhydride levels rise. The inclusion of hydrophilic succinate groups in starch dramatically increased viscosity (Xie 2005). The size of the starch granules, the agents utilized, and the reaction duration all affect the efficiency of a succinylation reaction. Due to the wide availability of anhydride molecules for starch molecules, studies demonstrate a linear increase in the DS corresponding with a rise in the succinic anhydride/starch proportion. The efficiency of the reaction is also influenced by the solvent. Only to a certain level is reaction time a restricting issue. The carboxylic acid ring of starch succinate is affected by pH, which has a substantial impact on its viscosity. At lower pH, the substituent group takes on an acid form, which is less hydrophilic than the salt form (Xie 2005).

When comparing these two reaction types, because of the pyridine usage succinylation does not prefer extensively in industry. Since, after the process pyridine must be separated from the product by distillation and the product must be washed. All these processes represent extra cost and time for the industry.

2.2.3.1.2 Etherification Reaction

Etherification is a process of chemical modification in which the hydroxyl groups are replaced by hydroxypropyl, carboxymethyl, and/or hydroxyethyl groups by forming an ether bond (R-OR). Unlike ester bonds like starch acetate, which deacetylate in alkaline conditions, ether linkages stay constant even at high pH. Etherification provides good viscosity stability to starch. Hydroxyalkyl starches, such as hydroxyethyl and hydroxypropyl, are primarily manufactured for industrial use. Because of its amorphous three-membered epoxide ring, propylene oxide is a non-symmetric epoxide and an extremely reactive chemical. The antisymmetric epoxide in the base is opened by attacking the sterically less restricted end of the epoxide, resulting in the formation

of 2-hydroxypropyl starch ether. In an alkaline medium, propylene oxide reacts with starch in a replacement, nucleophilic, bimolecular reaction. In an SN2 reaction, nucleophiles interact with oxiranes to produce ring-opened compounds. (Reactions 1 and 2) (Xie 2005). Figure 2.8 demonstrates the reaction mechanism of etherification

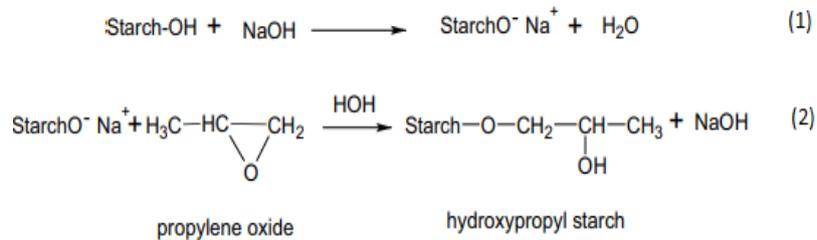


Figure 2.8. The reaction mechanism of etherification

(Souece: Xie 2005)

The internal bond arrangement of starch chains is disrupted when hydroxypropyl groups are substituted, resulting in a decrease in the amount of energy required to solubilize the starch in water. As a result, as the level of hydroxypropyl substitution increases, the pasting temperature of starch falls. The starch turns cold water swelling when the DS reaches a particular level. Cross-linking hydroxypropyl starches is commonly performed to increase viscosity long-term stability, weak pHs, and mechanical shear, as well as to provide the required texture (Xie 2005).

2.2.3.2 Depolymerization Reactions

With the depolymerization reaction, some properties like; using less energy needed to cook (because of the weaker granules), improving solubility, and lowering both hot-paste and gel viscoelastic properties (because of depolymerization). This allows for the addition of more starch to the solution. It enhances the ability to create films, can improve adhesiveness, improves the tendency to gel, and can create better gels are gained (Ojogbo 2020)

2.2.3.2.1 Acid Hydrolysis

The hydrolysis of a few of the glycosidic bonds of amylose and amylopectin in an aqueous slurry of corn starch at an acidic pH value and generally at a temperature of a little under 50°C for a fairly short period (one to several hours) results in acid-modified (thinned) starch (BeMiller 2019).

2.2.3.2.2 Dextrinization

Dextrin is a word used to describe depolymerized (degradation) starch compounds. By heating dried starch, a category of compounds known as starch dextrin is created, and they are also known as pyrodextrins. White dextrin, British gums, and yellow or canary dextrin are the three types of pyrodextrins. The starch is heated at a low temperature in the presence of an acid to produce white dextrin. British gums are created by heating starch in the presence of alkali at a reasonably high temperature. White dextrin is lighter in color than British gums. Highly converted products, such as yellow dextrin, are what they're called. They're manufactured by heating starch in the presence of an acid to relatively high temperatures. Throughout dextrinization, two types of reactions can occur. The first is glycosidic linkage hydrolysis, which occurs most often when an acid catalyst is applied. Transglycosylation is the second type of process, which involves the transfer of a part of a starch molecule to another starch molecule. Remoistenable glue, adhesives for producing paper tubes, and mining, foundry, and printing applications all use yellow dextrin and British gums. Crispiness additives for batters (in culinary goods), coatings for medicinal tablets, and textile finishing are all used for white dextrin (Chen 2018)

2.2.3.2.3 Oxidation

The broken glucosidic bonds and the oxidation of hydroxyl groups of starch to carbonyl and carboxyl groups are the principal effects of starch oxidation. Based on the oxidizer and the reaction parameters, carboxyl (COOH) and carbonyl (C=O) groups are added when depolymerization occurs. The only treatment in which a single modifying reagent generates two significant chemical alterations (depolymerization + carboxyl

group addition) is oxidation. Since about the early 1800s, different oxidants have been used to oxidize starch, including periodate, hypochlorite, hydrogen peroxide, permanganate, persulfate dichromate, and chlorite. Starch is modified by oxidant agents by generating new functional groups in the molecule. The use of hypochlorite or, more rarely, potassium permanganate for oxidation is an old approach that is still employed today (Tharanathan 2005). It entails the transformation of a primary hydroxyl group into a carboxyl group. Since oxidation is an exothermic reaction, it's important to keep the temperature of the reaction under control to avoid further polymer chain breakdown. Figure 2.9 shows the most common oxidation reactions.

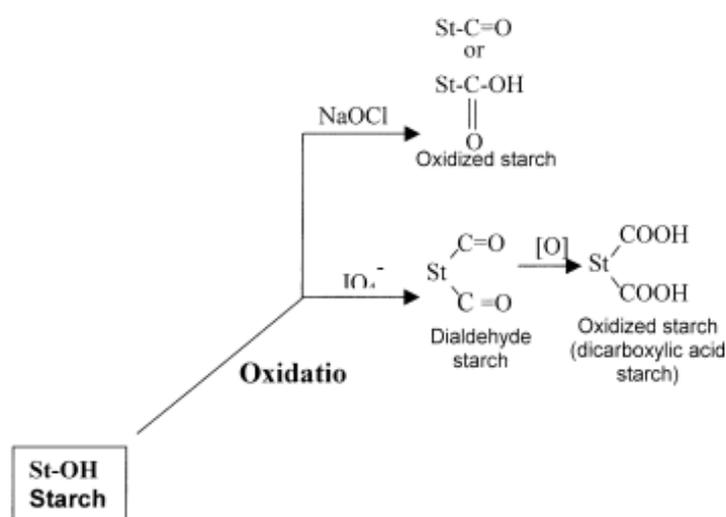


Figure 2.9. Common oxidation reactions

(Source: Tharanathan 2005).

Sodium Hypochlorite (NaOCl) and Perodite (IO_4^-) are mostly used oxidation agents, but because of the cost, in the industry mostly sodium hypochlorite is preferred.

Hypochlorite oxidized starches have enhanced color, decreased energy required to cook, hot-paste viscosity, diminished gel formation, and increased paste stability and adhesion as a result of the bleaching, depolymerization, and stabilization. Cleavage of polymer chains and oxidation of hydroxyl groups to carbonyl and carboxyl groups are the main processes of hypochlorite oxidation of starch. Principal hydroxyls (C-6), secondary hydroxyls (C-2, C-3, and C-4), aldehydic reducing end groups, and glycol

groups (cleavage of C-2–C-3 bonds) all oxidize at arbitrary. Due to the obvious huge number of hydroxyls at these sites, oxidations at C-2, C-3, and C-6 positions may have the most impact on starch characteristics (Xie 2005). Figure 2.10 demonstrates the formation of carbonyl and carboxyl groups during the hypochlorite oxidation process.

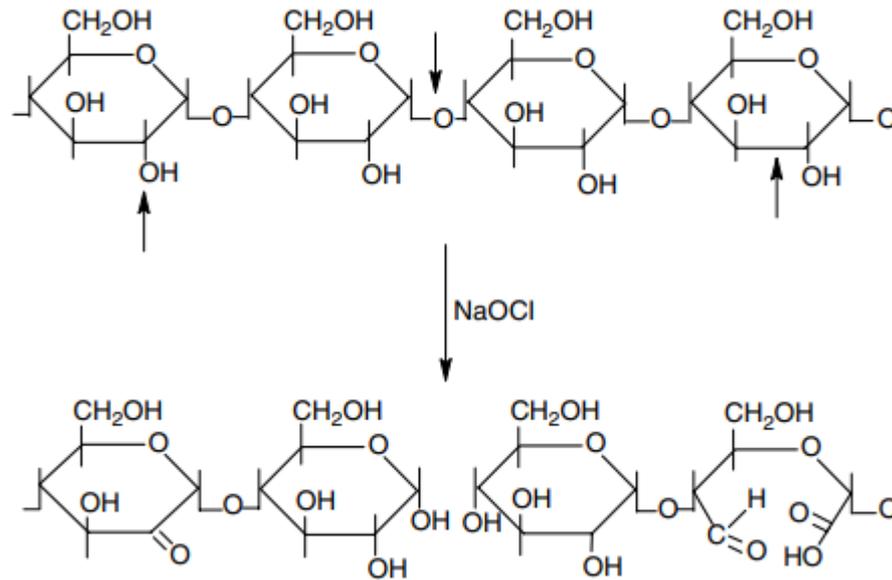


Figure 2.10. Oxidation with sodium hypochlorite

(Source: Xie 2005).

The depolymerization of amylose and amylopectin caused by the breaking of the glucosidic bond reduces the swelling ability and slurry viscosity. Processing with modest quantities of hypochlorite, on the other hand, has been shown to improve paste viscosity. The irregular formation of carbonyl and carboxyl groups throughout the chains lowers the gelatinization temperature, enhances solubility, and minimizes gelation. The carbonyl and carboxyl groups further lower the oxidized starch's thermostability, which produces browning. Because the bulkiness of the carboxyls and carbonyls sterically interferes with amylose's propensity to connect and retrograde, oxidized starches generate pastes that are clearer and more stable than natural starches (Masina 2017). The reaction is most quick at neutral pH, and it can be slowed down by reducing the pH and eliminating the chlorine that has been liberated. When it comes to

starch suspensions, a limited amount of carbonyl groups introduced during oxidation is favorable.

2.2.3.3 Crosslinking Reactions

Primary (6-OH) and secondary (6-OH) hydroxyls are found in starch (2-OH and 3-OH). These hydroxyls can react with a variety of multipurpose chemicals to form cross-linked starches. Cross-linking is used to avoid starch gelatinization or to limit the swelling of starch granules during cooking. A small amount of reagent is used to crosslink starch. In granules, starch granules are long-chain polymers that occur nearby. Reactions involving small amounts of a multipurpose reagent can join starch molecules. Just food starches are used for cross-linking. Granules are strengthened by crosslinking. Cross-linking is used to produce granules more resistant to the high temperatures, high shear, and acidic conditions that starch components are exposed to during the processing of processed foods (BeMiller 2019). For food starches, three types of cross-linking have been accepted. Phosphate ester is the most often generated cross-linked product, and phosphorus oxychloride (phosphoryl chloride, POCl_3) is the most commonly employed chemical for this reason. The adipic acid diester, which is formed by reacting starch with acetic-adipic mixed anhydride, is the second type of authorized cross-link. The glyceryl diether, which is formed by reacting epichlorohydrin with glycerol under alkaline circumstances, is the third type of cross-link (BeMiller 2019).

For some intended properties, one modification is not enough to get these properties. The required qualities can be achieved by combining two (dual treatment) or even more procedures. A combination of physical and chemical or chemical and chemical might be used to increase the processability of starch. Physical modifications reduce the molecular size and change the shape of molecules thanks to these changes chemical modifications occur easily in a shorter time (Xie 2005). When two different chemical modifications are used in order, different characteristic groups are substituted with starch's -OH groups or/and bonds are broken. With these double modifications starch gain different properties for the industry. When the dual modification and single modification compared, dual modifications are more effective than single modifications (Masina 2017). The most common modification combinations are substitution and crosslinking, and substitution and depolymerization. Before undergoing etherification

or esterification, native starch can be acid-modified or oxidized to produce lower viscosity starch ethers or esters. To create products with the required viscosity, starch esters or ethers can be depolymerizable by dextrinization, acid modification, or enzyme modification. When compared to the source starches, the starch pastes of these dual-modified derivatives have a reduced viscosity and a lesser tendency to retrograde (Swinkels 1998).

To reduce viscosity, and gelling temperature for the adhesive industry oxidation and acetylation reactions were selected to modify corn starch.

CHAPTER 3

LITERATURE SURVEY

The literature studies on chemical modification of starch by oxidation, acetylation and combination of both methods have been reviewed in this section.

In the study of Zhou and coworkers (2016), the effect of oxidation with different active chlorine content (0, 0.1, 0.2, 1.0, 2.0, 3.0, and 4.0 g of active chlorine for 100 g of starch) on potato starch's functional properties and its digestibility were investigated at 35°C and pH 9.50. They found that carbonyl and carboxyl content increased with increased active chlorine. Furthermore, the carboxyl content increased considerably quicker than the carbonyl content; this behavior was viewed as the hydroxyl groups on the starch molecules being oxidized first to carbonyl groups and subsequently to carboxyl groups. When polarized light microscopy (PLM) and scanning electron microscopy (SEM) images were investigated, the natural starch granules (Figure 3.1) in the SEM images were oval and spherical, with big and tiny granules and smooth surfaces absent of cracks. The surfaces and interior structures of the oxidized starch granule were different from those of the native starch granules. Pores form on the surfaces of the starch granules as the number of active chlorine increases, and their shapes become random. And also, the polarization cross of the PS granules is noticeably diminished as the oxidant concentration increases.

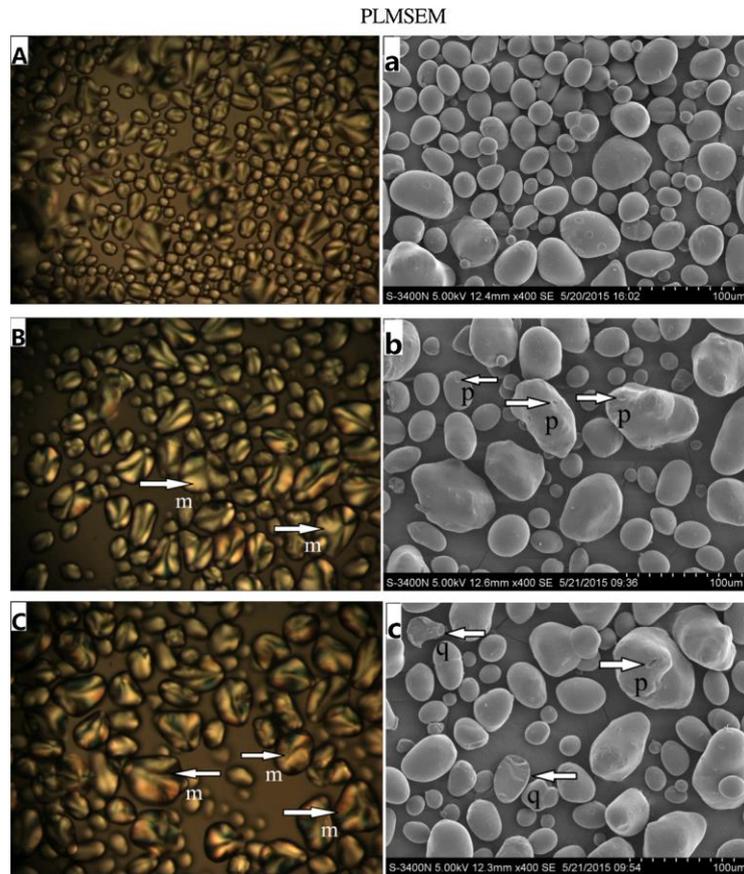


Figure 3.1. Morphologies of starch in PLM and electron SEM. Native starch (A, a), starch oxidized with 2% active chlorine (B, b), and with 4% active chlorine (C, c), m: polarization cross weakened, p: pores, q: crack.

As demonstrated in the figure, cross-polarization decreased in the PLM analysis of oxidized potato starch, although it could still be observed hence, oxidation mostly affects the amorphous areas of potato starch granules. And also, when compared to the native samples, the final and peak viscosities of the oxidized starch did not vary considerably when treated with low oxidant concentrations (0.1 and 0.2 g of active chlorine for 100 g of starch) but reduced dramatically from 7279 cP to 3934 and 3734 cP when treated with high oxidant concentrations (3.0 and 4.0 g of active chlorine for 100 g of starch) because of the depolymerization reaction. This result is important for the viscosity reduction objective.

In the study of Sangseethong and coworkers (2005), 40% cassava starch/water mixture was oxidized with sodium hypochlorite (3% active chlorine) at different reaction medium pHs (7.0, 8.0, 9.0, 10.0, and 11.0) for various reaction time (after

feeding) from 30 to 300 min at 30°C with 20 min feeding time. They considered the results, with increasing pH, the rate of oxidant consumption was reduced. The oxidant disappeared quickly during oxidation at pH 7 and 8. After 60 min, the reaction mixture contained less than 0.1 % oxidant as shown in the Figure 3.2.

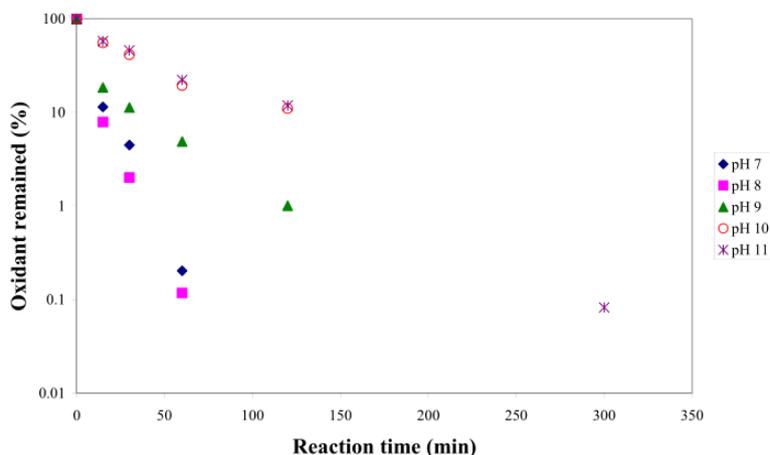


Figure 3.2. Consumption of sodium hypochlorite during oxidation reaction with different pH values

The production of carbonyl groups was found to be maximum as 0.4 % at pH 7 and declined with the reaction medium pH increased. The quantity of carbonyl group appeared to have little influence on reaction time; regardless of reaction pH, carbonyl amounts remained constant after just 30 min. of reaction time. The greatest quantity of carboxyl groups as 0.80% and 0.89% and was formed at reaction pHs 8 and 9; at these reaction pHs, the rate of carboxyl group production was very quick, remaining constant after just 30 min of reaction time. At pH 10 and 11, on the other hand, the quantity of the carboxyl group steadily rose with reaction time. The minimum solution viscosity as 11 mPa.s of starch was obtained after oxidation at pH 7, demonstrating that oxidative breakdown of starch molecules would dominate at this pH. The oxidation of starch at pH 8, 10, and 11 created a starch solution with intermediate viscosity (30-50 mPa.s) values, however, starch oxidized at pH 9 produced the maximum solution viscosity (70 mPa.s), meaning that depolymerization would occur at a considerably slower speed.

In the study of Garrido and coworkers (2014), cassava starch was modified with sodium hypochlorite (NaClO) at different concentrations (0.8, 2.0, and 5.0 % active chlorine %) and physicochemical properties were investigated. 40% starch water mixture was oxidized at pH 9.5 and 35 °C for 30 min feeding time and 50 min reaction time. When they considered the results, because acidic or neutral conditions and high temperatures (above 40°C) promote the transformation of hypochlorite to chlorate, which is inefficient in promoting oxidation, the oxidative medium must be alkaline. The Carboxyl group's percentage increased with the increasing amount of NaClO. The infrared spectra of all native starches and modified starches are given in Figure 3.3. Native starch shows 2,900–3,000 cm⁻¹ bands range show C-H stretching, C–O, and C–C stretching with contribution from C-OH stretching are shown 1,163, 1,150, 1,124, and 1,103 cm⁻¹ band ranges. C-OH and CH₂ deformations are responsible for the areas 1,077, 1,067, 1,047, 1,022, 994, and 928 cm⁻¹. The C-O-C contain in a six-carbon glucose ring at 1,150–1,085 cm⁻¹, and these bands will alter with the crystal structure of the starch samples. These bands can be noticed in the FTIR spectra of all starch samples. The oxidation of starch macromolecules generated structural changes; however, these are too small to be shown by FTIR spectroscopy because of overlapping. The area between 800 and 1,500 cm⁻¹ is characterized by significant overlapping, making it more difficult to identify specific bands.

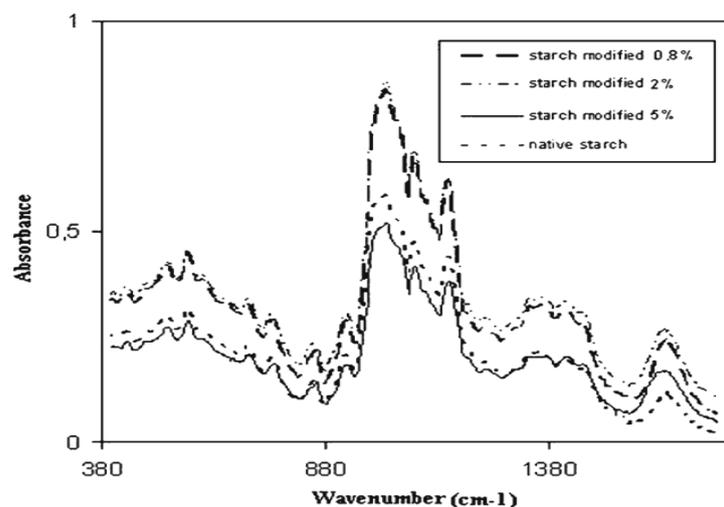


Figure 3.3. FTIR Spectrum of native and modified starch

In the study of Sandhu and coworkers (2008), waxy and natural corn starch oxidation with sodium hypochlorite that has %1 active chlorine was realized to observe the effect of the amylose content of starch on oxidation. All reactions were taken place at 35°C at pH 9.20 for 30 min feeding and 50 min reaction time. They observed that carbonyl and carboxyl content of normal oxidized starch was higher than waxy one because of the higher linear amylose content. It makes more favorable it for oxidation. The solubility of ordinary corn starches increased significantly after modification. The improvement in solubility upon oxidation is caused by depolymerization and structural degradation of the starch molecules. In contrast, oxidized waxy corn starch was less soluble than its natural equivalent starch. This might be because cross-links inhibited the amylopectin molecule from leaching out. After equal storage times, the light transmittance of the oxidized starch slurry was greater than native starches. This demonstrated that oxidized starches have minimal levels of retrogradation in contrast to native starches. When compared to regular corn starch pastes, both native and oxidized starch pastes demonstrated greater transmittance than waxy corn. The improvement in transmittance following oxidation is caused by the chemical replacement of hydroxyl groups in starch molecules with carbonyl and carboxyl functional groups. This creates repulsion between starch molecules and appears to diminish interchain interaction, allowing for better transmission. Also, they found, the quantity of heat required in the gelatinization process, represented by the gelatinization enthalpy (ΔH_{gel}), was lowered following oxidation in both waxy and natural corn starches. The ΔH_{gel} of oxidized waxy corn starch is higher than ordinary corn starch. This indicates that the double chains that unwind and dissolve during gelatinization are closely connected with the original granule. Furthermore, oxidized starches have lower transition temperatures (Gelatinization onset temperature T_o , Gelatinization peak temperature T_p , Gelatinization conclusion temperature T_c) than their native equivalents. The declines in T_o , T_p , and T_c of starches during oxidation might be attributed to the breaking of the starch granules.

In the study of Kesselmanns and coworkers (2004), waxy potato starch oxidation with sodium hypochlorite was studied at different pHs. They investigated the effect of pH on solution viscosity and its stability. According to their study, the oxidation process gave different dominant effects at different pH values as shown in Table 3.1.

Table 3.1. Effect of pH on oxidation process

	pH during oxidation		
	7.0–7.5	8.0–9.0	9.5–10.5
Degradation	+++	+	++
Carboxyl groups	+	+++	++
Carbonyl groups	+++	++	+

As shown in the Table 3.1, the oxidation process had a great degradation effect at pH 7.0-7.5 but in this range, the carboxyl group percentage was very small thus the stability of the starch solution was very low. And also because of the carbonyl group, the stability of the solution was affected negatively, and the solution color became a yellow-brown color which is an unwanted situation. At pH 8.0-9.0 degradation process was quite low thus reduction of solution viscosity was not observed properly although the viscosity is stable thanks to the carboxyl groups. In between 9.5-10.5, degradation carbonyl, and carboxyl groups distribution was optimum. Thus, viscosity was very stable.

In the study of Fonseca and colleague (2015) effect of different active chlorine amounts (0.5, 1.0 and 1.5 g/100g) was studied on the pasting, morphological, physicochemical, gel texture, and thermal properties of potato starch. They observed that, the carbonyl percentage of potato starch increased following oxidation by sodium hypochlorite, however there was no notable change in the carbonyl percentage of modified starches using 1.0 and 1.5 g/100 g of active chlorine. The shape of the starch granules was unaffected by oxidation. The variations in viscosity of starch slurry showed the pasting characteristics. As shown in Table 3.2, when compared to the native starch, oxidation of starch at lower concentrations (0.5 and 1.0 g/100 g active chlorine) enhanced viscosity while oxidation at high levels (1.5 g/100 g active chlorine) decreased viscosity. The increment of viscosity in low-oxidation starches may be owing to incomplete depolymerization of the starch. The oxidation process enhances depolymerization of the starch molecules amylopectin and amylose that reduces retrogradation and viscosity. Because carbonyl and carboxyl radicals are bigger than hydroxyl radicals, they increase the distance between the amylose chains, limiting molecular contact and hence reducing retrogradation.

Table 3.2. Viscosity changes of potato starch with different oxidant concentration

Active chlorine (g/100 g) ^a	Pasting temperature (°C)	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)
Native	61.1a	504.2c	359.7b	173.8c	29.3a
0.5	61.1a	642.9a	397.5a	275.5a	30.0a
1.0	60.5a	560.6b	392.6a	195.8b	27.9a
1.5	61.7a	324.6d	190.3c	151.8d	17.5b

^a Different letters in the same column differ statistically ($p < 0.05$).

Singh and colleagues (2004) studied the acetylation of corn and potato starch with different acetic anhydride (AA) amounts (2/4/6/8/10/12 g AA) at 25°C at pH 8 with 50% dry content to observe the difference in starch type. In reaction, acetic anhydride was fed dropwise (1 ml/min) meanwhile reaction pH was kept at 8. After feeding, the reaction was taken place for 10 min.. When considering the results, SEM showed significant diversity in size and shape in the granular structure of native potato and corn starches. Potato powder particles vary in size from 15–20 mm for tiny granules to 20–45 mm for big granules. Corn starch granules vary in size from 5–7 mm for tiny granules to 15–18 mm for big granules. The particles of native potato starch were found to be oval, smooth, irregular form. The granule of native corn starch seemed to be less smooth, rounded, and angular form as demonstrated in Figure 3.4.

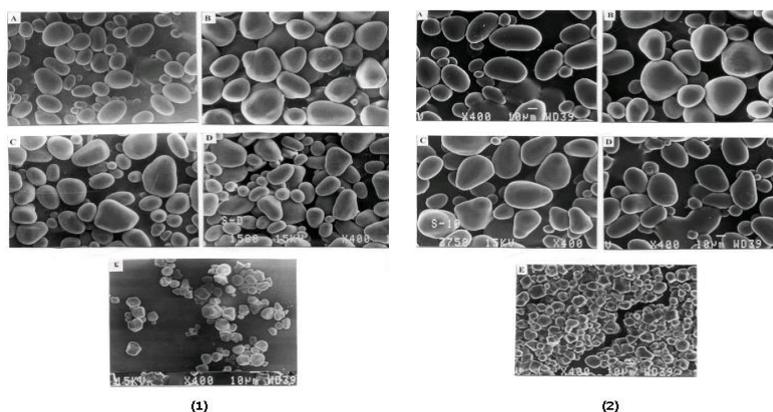


Figure 3.4. SEM images of native (1) and acetylated (2) corn (1E-2E) and potato starch (1A-1B-1C-1D-2A-2B-2C-2D)

The acetylation reaction causes particle fusion, which was particularly prominent in potato starches with tiny granules. For acetylated starches, the particle surface was found to be rather rough. The introduction of acetyl groups on the starch molecules, which led to an increase in hydrogen bonding, might be linked to the fusing of starch granules following acetylation. And also, the percent acetyl content and degree of substitutions (DS) of the acetylated corn and potato starches varied significantly. In the current study, acetylated corn starch had a lower percent acetyl content and DS than potato starches, which might be related to the corn starch particles' more solid structure and greater lipid content. Because potato powder particles are more brittle than maize starch, they may have been impacted more severely during acetylation. Two native and acetylated corn and potato starches had considerably different light transmittance percent. Native potato starches had much greater light transmittance than native corn starches. The variations in light transmittance of native starch pastes might potentially be related to changes in particle size distribution among cultivars. The light transmittance of the acetylated starch solution was much greater than their native equivalent starches and when DS was increased, transmittance was also increased. Native potato and corn starches had thermal decomposition peaks around 60 and 80°C, whereas acetylated starches had peaks between 55 and 75°C. The transition temperatures (T_o , T_p , T_c) and gelatinization enthalpy (ΔH_{gel}) of starches derived from native potato and corn starches vary considerably. Potato starch had higher ΔH_{gel} and smaller transition temperatures than corn starch. These variations in corn and potato starch may be related to variations in starch granule shape, amylose concentration, and gelatinization temperature. After acetylation, T_o , T_p , T_c and enthalpy ΔH_{gel} of all starches decreased but potato starch exhibited a greater change in this variable. The greater the DS of the starch, the greater the reduction in thermal parameters.

Han and coworkers (2012) studied the optimization of corn starch acetylation for different reaction times (60-240 min), temperatures (25-55°C), amounts of acetic anhydride (4-28%) and dry starch content (27-62%), at different pHs (6-11). All parameters were effective on the degree of substitution (DS) and reaction efficiency (RE) as shown in Fig 3.5. DS and RE increased as the reaction time rises to 150 min. and then drop. Because the esterifying agent is entirely consumed and the rate of hydrolysis of acetylated starch is quicker than the rate of production, so DS and RE declined. DS and RE enhanced when reaction temperature changed from 25 to 30°C.

The higher temperatures, in general, enhanced the diffusion of esterifying agents and swelling of starch granules, which destroyed the crystalline areas of the starch granules. Because esterification is an exothermic process, higher temperatures are harmful to the adsorption of the esterifying agent by the starch and the development of effective reaction sites. As a result, DS and RE slowly reduce as the temperature rises. Higher water content aids in the diffusion, dissociation, and adsorption of esterifying agents, which is beneficial to the process. Higher V_{H_2O}/m_{starch} ratio may cause various side reactions, such as the degradation of starch acetate, resulting in a rearward movement in the chemical equilibrium. The DS increased as the $m_{acetic\ anhydride}/m_{starch}$ ratio increases. The reaction efficiency decreases when the $m_{acetic\ anhydride}/m_{starch}$ ratio rises. On the other hand, DS rises, which might be due to greater interaction between the starch granules and the acetic anhydride. A higher $m_{acetic\ anhydride}/m_{starch}$ may result in acetic anhydride hydrolysis. When the pH of the reaction medium was increased from 7.2 to 8.2, DS and RE was improved. As the pH became higher, NaOH permeated into the amorphous and crystalline areas, breaking the hydrogen bonds. It promoted the esterifying agent's diffusion and absorption into the starch granular structure. The higher the pH of the reaction mixture, the better the hydrolysis of acetic anhydride, and hence reduce the quality of esterifying agent in this process as shown in the Figure 3.5.

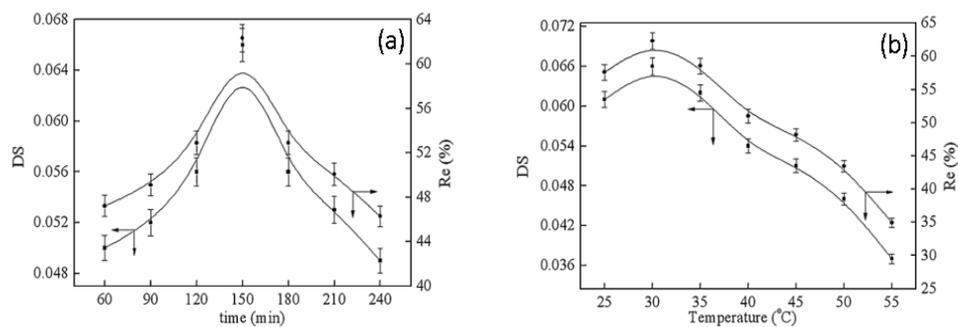


Figure 3.5. Effect of esterification reaction condition (a) time, (b) temperature, (c) amount of acetic anhydride, (d) water, (e) pH on the degree of substitution (DS) and reaction efficiency (RE) ((cont. on next page)

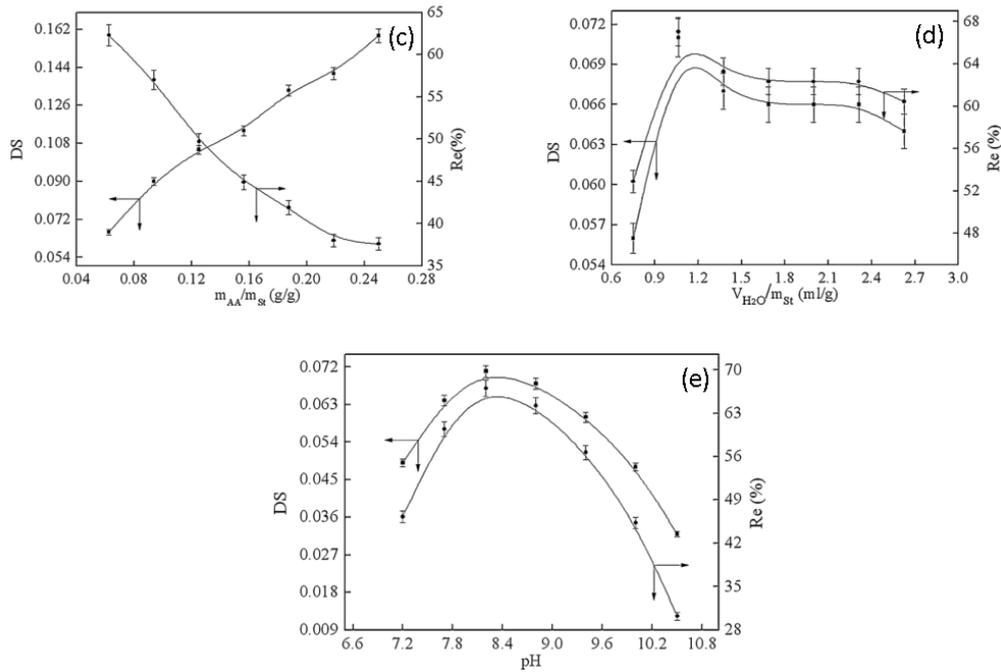


Figure 3.5. (cont.)

Figure 3.6 shows FTIR spectra of natural and modified corn starch. The band at 1158, 1083, and 1013 cm^{-1} in the natural corn starch were linked to C-O bond stretching. Intramolecular and intermolecular hydrogen-bonded hydroxyl groups were assigned the band stretch of approximately 3400 cm^{-1} . The scissoring of two O-H bonds of absorbed water molecules is responsible for the band at 1646 cm^{-1} . There are numerous more absorption bands at 576, 763, 857, and 929 cm^{-1} owing to the complete anhydroglucose ring stretching vibrations. Acetylated corn starch exhibits several additional bands in 1733, 1375, and 1252 cm^{-1} ascribed to carbonyl C=O stretch vibration, CH_3 symmetry deformation vibration, and carbonyl C-O stretch vibration, respectively, when compared to native corn starch. These new bands demonstrate that native corn starch has been esterified.

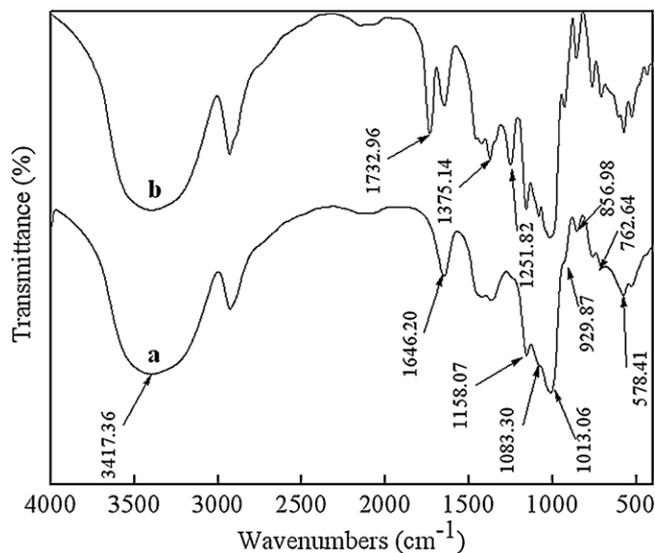


Figure 3.6. FTIR spectroscopy of native (a) and acetylated corn starch (b)

In addition, with increasing DS, the endotherms move to a lower temperature and the peak regions drop significantly. T_o , T_p , T_c , and ΔH_g values all drop. Gelling temperatures are generally related to the degree of the crystallinity of starch molecules, and gelatinization enthalpy offers an overall assessment of crystallinity and is an indicator of the damage of molecular chains inside the granule; the lower ΔH_g of ACS indicates a smaller percentage of arranged structures or lower stability of the crystals. And, the gel transparency of acetylated corn starch samples is much worse than that of natural corn starch. The increase in transparency following acetylation can be explained by the higher swelling of the acetylated corn starch, which enables light to flow through the grains rather than being reflected. This is due to starch granules dissociating and the granules' capacity to reflect light diminishing. The paste brightness improves as the DS grows.

In the study of Pietrzyk and colleague (2014), different oxidation ratios of corn starch were investigated for effect on the oxidized starch acetylation process and its rheological and physicochemical properties. In this study, corn starch was modified with sodium hypochlorite (1.0, 2.0, 4.0% active chlorine) at room temperature and pH 10. Obtained oxidized starch was acetylated with acetic anhydride at room temperature and pH 8-9. The findings are given Table 3.3. They found that the carbonyl and carboxyl group contents of starches improved as the quantity of oxidant applied in the procedure

increased. The maximum acetyl group concentration was found in acetylated oxidized starches oxidized at level 2.0%. This was attributed to the significant depolymerization of starch molecules and the degradation of starch granule morphology caused by oxidation, which allowed the chemical to penetrate deeper into the granules when compared to native starch and that oxidized at level 1.0%. The decreased acetylation of starch oxidized at level 4.0% compared to level 2.0% was explained by a reduced number of hydroxyl groups coming from their prior oxidation to carboxyl and carbonyl groups. They hypothesized that after acid treatment, the breakdown of amylopectin and amylose, as well as the self-association, and realignment of starch polymers, might lower the number of acetylation target sites

Table 3.3. Carboxyl, carbonyl content, and acetyl groups in modified starch

Starch	Carboxyl groups (%)	Carbonyl groups (%)	Acetyl groups (%)
Oxidised I	0.01 ± 0.00	0.02 ± 0.00	-
Oxidised II	0.04 ± 0.01	0.04 ± 0.01	-
Oxidised III	0.43 ± 0.10	0.10 ± 0.01	-
Acetylated	-	-	1.66 ± 0.02
Acetylated oxidised I	-	-	1.54 ^a ± 0.01
Acetylated oxidised II	-	-	1.71 ± 0.01
Acetylated oxidised III	-	-	1.58 ^a ± 0.01

The same small letters indicate values that are not significantly different at $\alpha = 0.05$.

The change in thermodynamic properties of starches were shown in Table 3.4. The transition temperature was lowered by oxidation (T_o). Acetylation reduced the T_o temperature by 4–5 °C for both natural and oxidized starch. The enthalpy of transition (ΔH) of all modified starches achieved in their study was lower than that of the natural starch. The acetylation process reduced this characteristic even further in oxidized starch. The decrease in ΔH may be attributed to the breaking of intermolecular interactions responsible for the crystallinity of amylopectin as a result of acetyl group entry into starch molecules.

Table 3.4. Thermodynamic characteristics of native corn starch and modified

Starch	T_0 (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J/g d.m.)
Native	66.1 ± 0.2	70.9 ± 0.2 ^{ab}	74.9 ± 0.5 ^a	8.7 ± 0.5	20.6 ± 0.3
Acetylated	60.8 ± 0.2	66.4 ± 0.1	71.0 ± 0.2	10.2 ± 0.4 ^a	15.7 ± 0.1
Oxidised I	65.7 ± 0.1 ^a	70.7 ± 0.3 ^a	75.3 ± 0.1 ^a	9.5 ± 0.1 ^b	16.6 ± 0.1
Acetylated oxidised I	61.7 ± 0.2	67.1 ± 0.3 ^c	72.8 ± 0.1 ^b	11.1 ± 0.3 ^c	11.8 ± 0.2
Oxidised II	65.9 ± 0.2 ^a	71.2 ± 0.1 ^b	76.0 ± 0.1	10.1 ± 0.1 ^{ab}	14.0 ± 0.2 ^a
Acetylated oxidised II	61.1 ± 0.2	66.9 ± 0.2 ^{c-d}	72.6 ± 0.1 ^b	11.6 ± 0.2 ^c	13.7 ± 0.2 ^a
Oxidised III	63.8 ± 0.1	69.7 ± 0.1	75.0 ± 0.1 ^a	11.2 ± 0.2 ^c	13.8 ± 0.1 ^a
Acetylated oxidised III	59.6 ± 0.1	66.5 ± 0.2 ^d	74.0 ± 0.1	14.4 ± 0.2	10.1 ± 0.2

The same small letters indicate values that are not significantly different at $\alpha = 0.05$.

As the amount of starch oxidation increased, the intrinsic viscosity of the solution decreased from 220 cm³/g to 48 cm³/g. Acetylation seems to have no significant effect on viscosity. The reduction in viscosity caused by modification might be attributed to the significant depolymerization of starch polymers and the subsequent decrease in molecular weight.

The scope of the thesis study was determined as follows after detailed revision of the literature on the topic. Modification conditions were selected for oxidation as 40% dry starch content, at room temperature, and pH 10 with 30 min feeding and 50 min reaction time. For the acetylation, process conditions were selected as 43% dry oxidized starch content, at room temperature, and pH between 8-9 with 1 ml/min feeding and 15 min reaction time. All selections were made by taking into consideration the prevention of all modifications from side reaction and obtaining optimum carbonyl, carboxyl, and DS values for application toward adhesives for optimum solution viscosity and gelling enthalpy and temperature.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

For the synthesis of oxidized starch acetate, corn starch, sodium hypochlorite (NaOCl, active chlorine 15-16%), acetic anhydride (99.5% purity), sodium hydroxide (2N), hydrochloric acid (2N), and distilled water were used. Corn starch was delivered by Sunar Mısır, sodium hypochlorite was purchased from Albar Kimya, acetic anhydride, sodium hydroxide, hydrochloric acid were purchased from Merck.

4.2 Synthesis of Oxidized Starch Acetate

The starch oxidation process was carried out following a method described by Pietrzyk 2019. Starch (40% water solution) was modified by different amounts of NaClO (6.0, 12.0, and 24.0 g Cl per kg of starch). 2N NaClO was fed to the starch solution while pH was kept at 10 for 30 min. After that, the reaction was continued for 50 min. further. At the end of the reaction solution's pH was adjusted to 7 with 2N HCl. All steps were carried out at room temperature. The modified starch was washed with distilled water two times by using filtered paper and funnel, dried at room temperature for 48 h.

Acetylation was performed as follows. 40% water solution of unmodified or oxidized starches were prepared at room temperature and the acetic anhydride was added drop-wise (1 ml/min) to the starch solution using a peristaltic pump. In the meantime, the solution pH was kept between 8 and 9 by adding 2N NaOH solution. The reaction was continued for 15 min after the feeding. At the end of the reaction pH of the starch solution was adjusted to 5.2–5.6 using 2N HCl and the modified starch was washed two times with distilled water by using filtered paper and funnel, dried at room temperature for 48 h. Different amounts of acetic anhydride addition, 7.50, 15.0, and 30.0 ml, were tested. Figure 4.1 shows the configuration of the experimental setup.

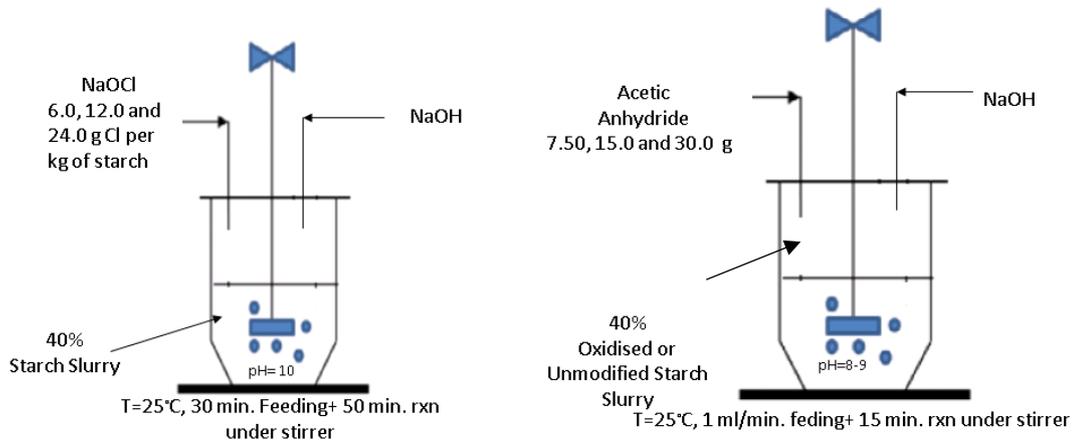


Figure 4.1. Experimental Configuration of (a) oxidation and (b) acetylation processes

Active chlorite content was measured as follows. Sodium hypochlorite was suitably diluted, and a sample of 10 mL was put in an Erlenmeyer flask, along with 30 mL of acetic acid 1:2 (v/v), and 30 mL of potassium iodide (10%, w/v). This solution was titrated with 0.1 M sodium thiosulphate to a bright yellow turned colorless. The active chlorine percentage (ACI %) was calculated by using the following equation.

$$\text{Active Chlorine \%} = \frac{(V \times fc \times 0.3722)}{(Vs \times d)} \times \frac{100}{10} \times 0.953 \quad \text{Eqn (1)}$$

(1)

Where:

V= 0.1 M thiosulphate solution volume (mL)

fc= correction factor of the 0.1 M thiosulphate solution is 1

Vs= Volume of the sample in mL

d= density of the sodium hypochlorite in g L^{-1}

4.3 Characterization of Oxidized Starch Acetate

4.3.1 Effectiveness of Oxidation and Acetylation Processes

The effectiveness of modifications was measured by acid-base titration to observe changes in carbonyl and carboxyl groups in the oxidation process, acetyl groups in the acetylation process.

For measurement of carbonyl groups, 2 grams oxidized starch and 100 ml distilled water were mixed and heated up to gelatinize temperature. In the gelatinization state, it was kept for 20 min and cooled down to 40 °C. After cooling, the solution pH was adjusted to 3.20 with 0.1 N HCl and then 15 ml of hydroxylamine solution was added to the solution. After that the solution was stirred 4 h. at 40 °C. End of the 4 h., solution pH was adjusted to 3.20 with 0.1 N HCl to determine remaining amount of hydroxylamine. The same method was also applied to natural starch. By using the analysis data, carbonyl degree was calculated using Equation (2).

$$\% \text{Carbonyl (g/100g)} = \frac{(V_{\text{unmodified}} - V_{\text{modified}}) \times \text{Acid normality} \times 0.0028}{\text{Sample Amount (g)}} \times 100 \quad \text{Eqn (2)}$$

Where 28 represents carbonyl (-CO) groups MW

For carboxyl group's measurement, 2 g oxidized starch and 25 ml 0.1 N HCl were mixed for 30 min at room temperature. End of the 30 min. it was filtrated and washed with 400 ml of distilled water to remove excess chlorine. After washing, the cake was mixed with 300 ml distilled water and it was heated up to its gelatinized temperature and kept at this temperature for 15 min. After that 150 ml of distilled water was added to the solution and the pH of the solution was adjusted to 8.3 with 0.01 N NaOH addition. Spent NaOH was recorded. The same method was also applied to natural starch. By using the analysis data, carboxyl degree was calculated using Equation (3). The carboxyl content of the natural starch was found similarly.

$$\% \text{Carboxyl (g/100g)} = \frac{(V_{\text{modified}} - V_{\text{unmodified}}) \times \text{NaOH normality} \times 0.0045}{\text{Sample Amount (g)}} \times 100 \quad \text{Eqn (3)}$$

Where 45 represents carboxyl (-COOH) groups MW

The degree of substitution (DS) was determined as follow. 2 grams of acetylated oxidized starch was mixed with 40 ml distilled water, and a few drops of phenolphthalein were added to the mixture. After addition solution was titrated with 0.1 N NaOH until its color changed to pink. After obtaining the intended color, 25 ml 0.15 N NaOH was added to mixture, and it was kept under stirrer 1.5 h at room temperature. After that the solution was titrated with 0.1 N HCl till it becomes colorless. Spent HCl amount was recorded. The same method was also applied to natural starch. By using the analysis data, degree of substitution was calculated using Equations (4) and (5).

$$\% \text{Acetyl (g/100g)} = \frac{((V_{\text{unmodified}} - V_{\text{modified}}) \times \text{HCl normality}) \times 0.0043}{\text{Sample Amount (g)}} \times 100 \quad \text{Eqn. (4)}$$

$$\text{DS} = \frac{162 \times \text{Acetyl \%}}{(4300 - (42 \times \text{Acetyl \%}))} \quad \text{Eqn. (5)}$$

4.3.2 Thermodynamic Characteristics of Gelatinization by DSC

The thermodynamic characteristics of gelatinization were studied by using a differential scanning calorimeter DSC 200 (Hitachi, Japan). In an aluminum calorimetric pan, a starch–water (1:3) solution was sealed and permitted to stand for 24 h. The samples were then heated at a rate of 10 C/min at temperatures ranging from 20 to 100°C. As a control, an empty calorimetric pan was used. The temperatures at the peak (T_p), onset (T_o), end of the transition (T_e), and transition enthalpy ΔT ($T_o - T_e$) of starch weight were measured.

4.3.3 Granules Morphology Analysis

The surface morphologies of starch were observed by scanning electron microscope (SEM, FEI QUANTA 250 FEG) with magnification ranging from 1000X to 10000X under secondary electron (SE) and back scattered electron (BSE) detectors.

4.3.4 FTIR

The FTIR analysis was performed using an IRSpirit FTIR spectrometer with QATR-S attachment (Shimadzu, Japan). FTIR spectra were recorded at a resolution of 4 cm⁻¹ and with a total of 20 scans, and wavenumber range between 400 and 4000 cm⁻¹

4.3.5 UV-VIS Spectrometry

The light transmittance of starch was analyzed with V-770 UV-VIS/NIR Spectrophotometer (Jasco, Japan). For this analysis, 2% starch distilled water solution was prepared. This solution was heated up to 90 °C under stirring and kept 30 min.. After this step, it was cooled to room temperature and conserved at 90°C for 4 days. After 4 days, light transmittance of starch was analyzed at 640 nm.

4.3.6 ¹H-NMR Spectra

¹H-NMR on a Nuclear Magnetic Resonance Spectrometer (AGILENT TECHNOLOGIES VARIAN 400) operated at 400 MHz. The product (25 mg) was dissolved in DMSO-d₆ (2 g). ¹H NMR spectra were acquired at 60 °C (64 scans) using a relaxation time of 1.0 s.

Also, the DS of acetylated starches might be determined using ¹H-NMR analysis. Acetylation caused the displacement of hydrogen bond to a lower magnetic field. The following equation might be used to determine the DS of acetylated starch with a low DS (DS ≤ 2.5) according to study of Teramoto and Shibata, 2006.

$$DS_{\text{low}} = \frac{4A}{3B+A} \quad \text{Eqn. (6)}$$

For higher DS (DS ≥ 2.5) of starch acetate can be obtained from the equation 7 Elomaa, 2004.

$$DS_{\text{high}} = \frac{7A}{3C} \quad \text{Eqn. (7)}$$

Here A is the total areas of methyl hydrogen areas at 2.01- 2.08 ppm; B is the total of areas of OH and H-1 proton for AGU unit share detected at ppm than 4.5 ppm; and C is the total peak areas of seven H in AGU unit seen at higher than 3.95 ppm.

4.3.7 Solution Viscosity of Starch

Solution viscosity of starch measured by Brookfield Viscometer DV2T (Brookfield, USA) with #4 spindle 100 rpm. Starch were dissolved in distilled water as 20% solid content at room temperature.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Effect of Oxidant Concentration

Effectiveness of oxidation and acetylation process were determined by carbonyl/carboxyl percentage and DS value calculated by Eqns. (2 to 5). The results obtained are given in Table 5.1. As shown in the Table, the oxidation process described as carbonyl and carboxyl percentages were increased with increasing active chlorine concentration of oxidant. This observation reached an agreement with other studies in literature for oxidized corn, potato, and rice starches (Kuakpetoon 2001; 2006; Martinez 2007), natural and waxy corn starches (Wang 2003) and banana starch (Sánchez, 2005). The carbonyl content of starch increased after oxidation with sodium hypochlorite, however, there is no notable change between the carbonyl percentage (0.0029-0.0030%) of modified starches with 6.0 and 12.0 g/100 g of active chlorine. The highest level was obtained with starches oxidized at level III.

Carboxyl groups content were always higher than the carbonyl groups because in the oxidation process hydroxyl groups of AGU in starch were oxidized carbonyl group firstly and transformed to the carboxyl groups to produce the final product in alkaline condition (pH 10) which provides to carboxyl groups production. Thus, the carboxyl group percentage is higher in oxidized starch. For investigation of the effectiveness of oxidant concentration on the acetylation process, natural starch was oxidized with different oxidant amount and acetylated with the same acetic anhydride amount. When the results were evaluated, the highest acetylation degree was obtained for Acetylated oxidized II. After this level acetylation groups started to decrease. This was attributed the substantial depolymerization of the polymer backbone and the degradation of crystalline structure caused by oxidation, which allows the oxidant to penetrate deeper into the granules when compared to native starch and that oxidized at the level I. The decreased acetylation sensitivity of starch oxidized at level III compared to level II might well be described by a reduced number of hydroxyl groups as a

consequence of their earlier oxidation to carboxyl and carbonyl groups. These results were in agreement with literature findings given by Pietrzyk 2014. Also these DS results were confirmed by ¹H-NMR results given in Table 5.1. A good agreement between two methods was observed. The reason for small differences in DS between titration and ¹H-NMR could be the personal measurement errors.

Table 5.1. Effect of oxidation concentration on corn starch oxidation and acetylation

Starch	Active Chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Carb-oxyl Groups (%)	Carbon-yl Groups (%)	Acetyl Groups (%)	DS by Titra-tion	DS by ¹ H-NMR
Natural Corn Starch	-	-	-	-	-	-	-
Oxidized I	6.00	-	0.77	0.0029	-	-	-
Oxidized II	12.00	-	1.11	0.0030	-	-	-
Oxidized III	24.00	-	1.33	0.0061	-	-	-
Acetylated Oxidized I	6.00	15.00	-	-	2.000	0.077	0.0079
Acetylated Oxidized II	12.00	15.00	-	-	2.275	0.089	0.0093
Acetylated Oxidized III	24.00	15.00	-	-	2.125	0.083	0.0089

Figure 5.1 demonstrates the FTIR spectra of natural, all oxidized, and all oxidized starch acetate starches with different oxidation levels. When FTIR characterization was performed to examine the effect of the amount of oxidizing agent on the chemical structure of starch, the peak of –OH stretching bonds can be observed in the wavenumber of 3000-3600 cm⁻¹ and 1650 cm⁻¹. For bending, in the wavenumber of 2950 and 1420 cm⁻¹ CH stretching and bending peaks have appeared for both natural and oxidized starches. Thus, there was no difference between natural and oxidized starch peaks for different oxidation amounts even with higher level carboxyl and carbonyl contents. This can be explained by the depolymerization due to oxidation. C=O stretching bonds, C-H bonds, and C-O stretching bonds from acetyl groups were formed at wavenumbers of 1735-1740 cm⁻¹, 1368 cm⁻¹, and 1234 cm⁻¹ in oxidized starch

acetates that undergo double modification after acetylation. 1735 cm^{-1} peak was effected by the acetylation level of starch and it increased with the acetylation agent amount. However, the intensity of OH peaks at $3000\text{-}3600\text{ cm}^{-1}$ was decreased. This showed that OH groups in AGU units transformed into acetyl groups. In this study, acetate peaks were obtained in all oxidized starch acetate and the highest peak intensity was obtained in Acetylated oxidized II. FTIR results also confirmed the effectiveness result of the oxidation process.

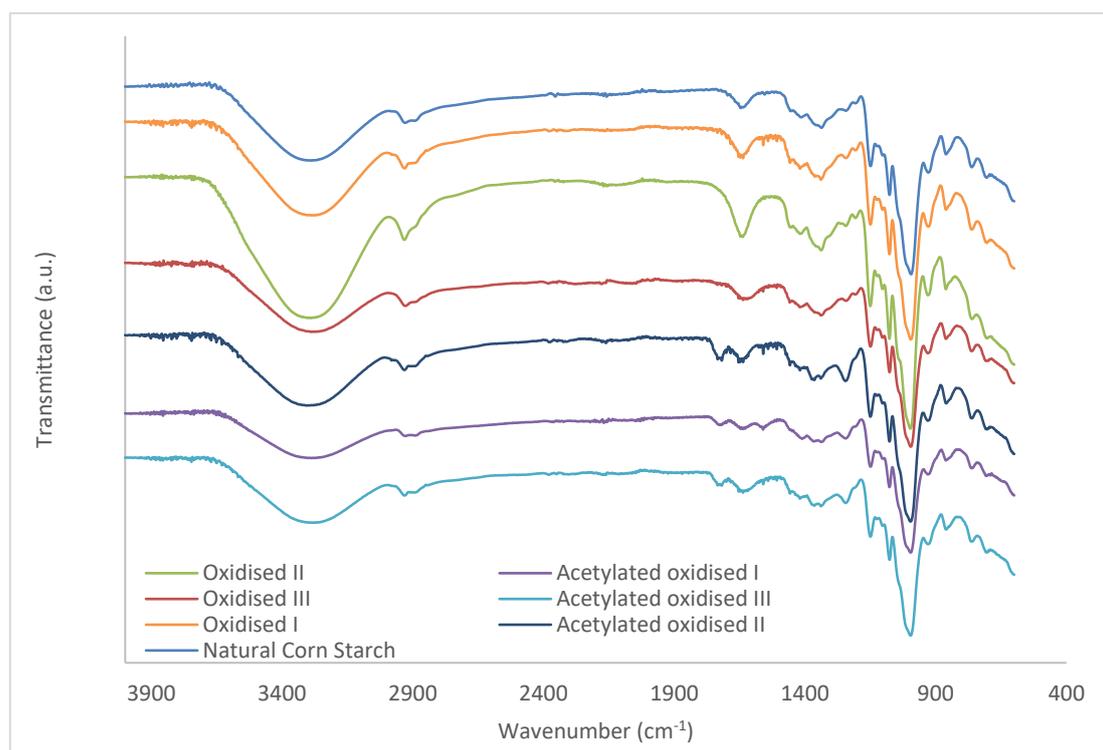


Figure 5.1. FTIR spectra of natural, oxidized, and acetylated oxidized corn starches for different oxidant levels

DSC analysis was performed to observe effect of different oxidant concentrations on thermal properties of modified starch. Figure 5.2 shows the DSC and DDSC (Differential DSC) curve of unmodified corn starch. The transition temperatures that are the temperature at the onset of gelatinization (T_{onset}), the temperature at peak of gelatinization (T_{peak}), and the temperature at the end of gelatinization (T_{end}) shown in the figure. ΔH values were calculated from the areas of these temperature peaks. Modified starch's transition and enthalpy values were tabulated in Table 5.2.

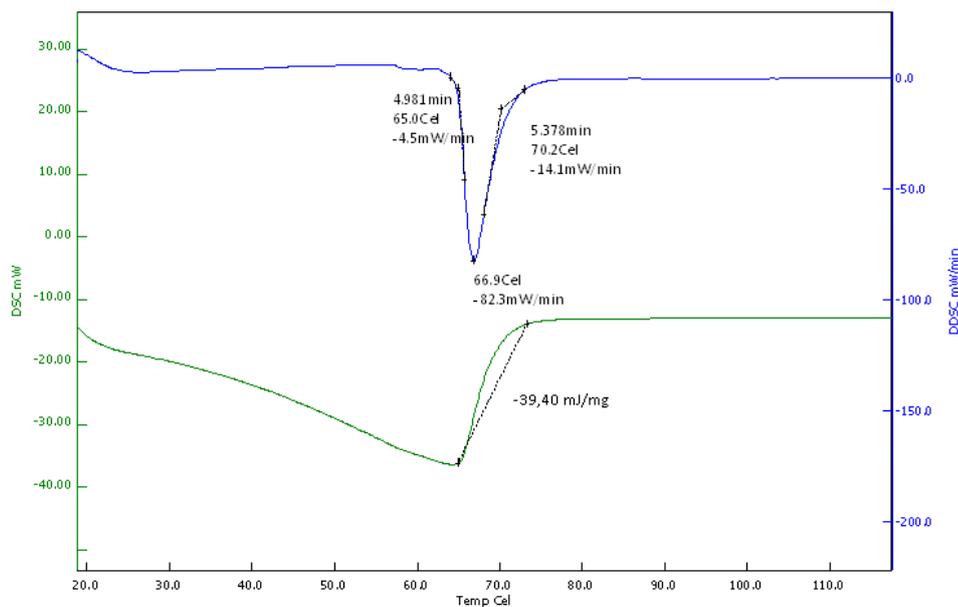


Figure 5.2. DSC and DDSC curve of unmodified corn starch

The transition temperatures could be decreased for energy savings. This is supported by a decrease in the enthalpy of gelatinization (ΔH) which indicates the energy required for the gelatinization process. It is an important parameter for large-scale production in the industry. Gelatinization enthalpies of natural and all modified starch are given in Table 5.2.

Table 5.2. Thermal properties of natural and oxidized and acetylated starch for different oxidant concentration

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	T _{onset} (°C)	T _{peak} (°C)	T _{end} (°C)	ΔH (J/g)
Natural Corn Starch	-	-	65.00	66.90	70.20	39.40
Oxidized I	6.00	-	64.50	67.40	80.90	30.60
Oxidized II	12.00	-	67.40	72.70	82.80	24.00
Oxidized III	24.00	-	65.00	67.40	71.10	4.41
Acetylated Oxidized I	6.00	15.00	68.80	72.40	80.20	12.20
Acetylated Oxidized II	12.00	15.00	54.60	66.40	70.80	10.90
Acetylated Oxidized III	24.00	15.00	71.50	76.50	81.70	4.24

As demonstrated in the table, natural corn starch had higher gelatinization enthalpy than modified starches. Gelatinization enthalpy of oxidized starch decreased with the increasing amount of oxidant. The lowest enthalpy value was found for the highest oxidant amount, Oxidized III. The reason for that might be due to the weakening of the intermolecular interactions essential for the crystal structure of amylopectin. Because of the presence of carboxyl groups in the oxidized starch granules, the crystalline structure of starch was broken down and transformed into the amorphous structure. The effectiveness of Oxidized III was also confirmed with this reduction. Furthermore, the starch particles modified with chemicals resulted in surface irregularities such as cracks and holes (as demonstrated SEM analysis) which enables water penetration into the granules. This situation could also decrease the gelatinization enthalpy. After acetylation of oxidized starch, enthalpy of gelatinization was found lower than only oxidized ones for all oxidized acetates. These decreases in ΔH indicate that the acetyl groups affect the amylopectin double helixes. Additionally, the introduction of large groups along the starch chain improves structural flexibility and helps to decrease modified starch gelatinization temperature. In addition, acetyl group introduction to starch molecules disrupts the crystallinity of starch. This could cause a reduction of enthalpy. Differences in the thermal characteristics of starches from various sources could be attributed to granule content, which might include the amylose/amylopectin ratio, remaining protein and lipids, amylopectin molecule structure, shape and dispersion of starch granules.

As shown in Table 5.3 light transmittance (%) value was affected by the modification type and applied modification content. Natural corn starch had a lower light transmittance value of 0.03%. Corn starch's poor light transmittance might be related to swelling light refraction. After oxidation with different oxidant concentrations, the light transmittance of starch increased but there was no considerable change with higher oxidation amount. Depolymerization of starch could be the cause of this increment. After acetylation of oxidized starch, an increase in light transmittances was observed remarkably. Because of the enhanced swelling, the acetylation modification enhanced the light transmittance in all starches. This enhancement might be attributed to the addition of hydrophilic substitution groups, which kept water in starch molecules to form hydrogen bonds, resulting in increased light transmission. These changes were the essential reason for the increment.

Table 5.3. Effect of oxidant concentration on light transmittance properties of oxidized and acetylated corn starch

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Light transmittanc e (%)
Natural Corn Starch	-	-	0.03
Oxidized I	6.00	-	7.46
Oxidized II	12.00	-	8.43
Oxidized III	24.00	-	9.27
Acetylated Oxidized I	6.00	15.00	28.96
Acetylated Oxidized II	12.00	15.00	37.85
Acetylated Oxidized III	24.00	15.00	58.23

SEM was used to examine the starches' surface structure. As shown in Figure 5.3 natural corn starch has a big, angled, and smooth surface typical of the corn starch. Although the modifications (oxidation and/or acetylation) did not affect the starch molecules, many fractures, scratches, and partial deformations of the shape of the particles were observed on their surface, which likely led to changes in their specific surface area. After the oxidation process, some scratches, small fractures, and folds were expected to be noticed, but they were not observed until 12 g Cl/kg starch. After this dosage, some scratches and fractures were observed on the surface of the oxidized starch. Fonseca 2015, El Halal 2015, and Kuakpetoon 2001 did not observe any surface changes in starch granules until a value in their studies. Other researchers observed that oxidation can impact the granule due to the existence of holes on the surface. Spier, 2013 modified maize starch and discovered that oxidation with an acid pretreatment caused pores on the granule surface. Vanier, 2012 found no differences in the shape of oxidized starch Granules with 0.5 and 1.0% active chlorine. However, when they oxidized the starch with 1.5 % active chlorine, the granules showed cracks on their outer surfaces. In our study, similar results were obtained. The high concentration of sodium hypochlorite of 24.00 g Cl/ kg starch could create cracks and porosity in the starch granules, but the low dose oxidant treatments that were Oxidized I and II had no discernible effect on the crystal structure of the oxidized starch.

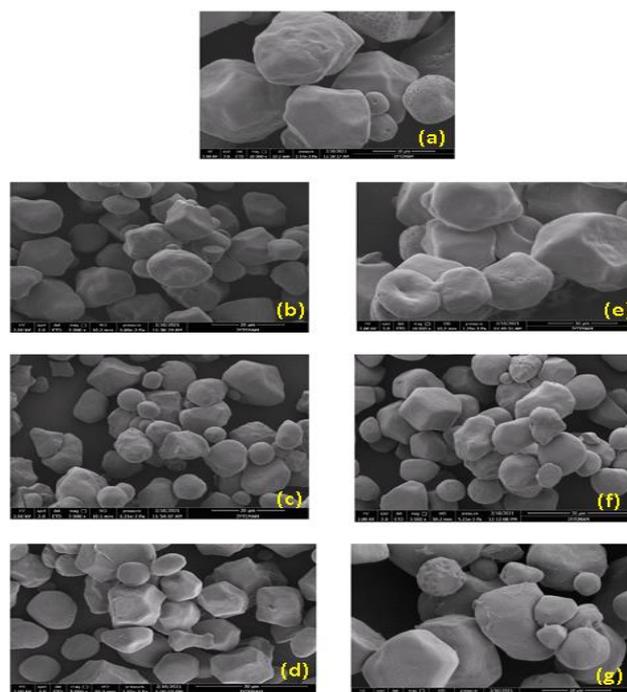


Figure 5.3. Scanning electron micrographs of natural and modified starches with different oxidant ratios. Native starch (a), Oxidized I (b), Oxidized II (c), Oxidized III (d) Acetylated oxidized I (e), Acetylated oxidized II (f), Acetylated oxidized III (g)

After acetylation of oxidized starch, the surface of oxidized corn starch granules changed and resulted in a quite rough surface than in native granules (Figure 5.3 e,f,g). These changes were observed in all doses of acetic anhydride and with increasing dosage, cracks and pores were formed. And also after the acetylation process, agglomerates formation was observed as shown in Figure 5.4b. The reason for that can be the insertion of hydrophilic groups to starch granules that held the water to form hydrogen bonds in starch molecules, which might be causing agglomeration of starch granules following acetylation.

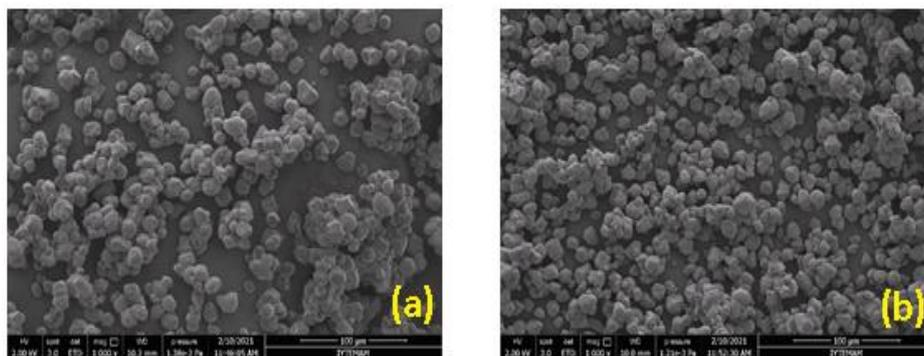


Figure 5.4. Scanning electron micrographs of oxidized I (a) and acetylated oxidized I (b)

All results demonstrated that the configuration of starch molecules is defined as semi-crystalline with crystalline and amorphous areas, the demonstrated effect is most largely because acetylation and oxidation target the amorphous region predominantly, trying to remove the area from the surface of the starch and causing granule rupture.

Solution viscosity of modified starch was one of the most important parameters in this study because of the processability of modified starch. In industry, all process has defined minimum and maximum process parameter and obtained starch had to be suitable for this process parameter. This parameter is directly proportionate to the molecular weight of starch. All solution viscosity results of natural and modified starch were tabulated in Table 5.4.

Table 5.4. Solution viscosity of natural and modified starches

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Solution Viscosity (cP)
Natural Corn Starch	-	-	218
Oxidized I	6.00	-	159
Oxidized II	12.00	-	126
Oxidized III	24.00	-	93
Acetylated Oxidized I	6.00	15.00	136
Acetylated Oxidized II	12.00	15.00	96
Acetylated Oxidized III	24.00	15.00	92

As shown in the Table 5.4 oxidation had a great effect on the solution viscosity of starch. With the increasing dosage of oxidant solution viscosity decreased. The lowest viscosity was observed in Oxidized III. The depolymerization process might cause the reduction. After the acetylation process of oxidized starch, reduction of solution viscosity was still observed but this reduction was not as much as observed in the oxidation. The rise in intrinsic viscosity observed following acetylation in corn starch would be most probably formed by the impact of neighboring starch polymer acetyl groups, which might result in an increase in overall molecular weights.

Natural and modified starches were analyzed by $^1\text{H-NMR}$ to observe proton signals. As shown in Figure 5.5 natural corn starch had ^1H shifts at 3.40-4.80 ppm that represent protons in AGU unit of starch. 3.30 ppm to H2, 3.67 ppm to H3, 3.35 ppm to H4, 3.07 ppm to H4 (end group), 3.59 ppm to H5, 3.64 and 3.46 ppm to H6, (Chi, 2008). With the oxidation and acetylation process, the hydrogen resonance frequencies of AGU differed from those of native starch. As demonstrated in the figure after oxidation, ^1H shifts at 5.10 ppm disappeared. This peak represents the protons in carbonyl and carboxyl units. After the acetylation process besides oxidation peaks, ^1H shifts at 1.90-2.10 ppm were observed. These peaks belong to methyl protons in acetyl groups. In the double modification of starch, both oxidation and acetylation peaks are clearly noticed. When considering the area of peaks at 5.10, with the increased amount of sodium hypochlorite (oxidized I, II, and III) area of peaks were calculated as 1.99, 2.37, and 3.87 respectively. The increasing amount of oxidant causes a more intense peak in $^1\text{H-NMR}$ spectra. After acetylation of these oxidized starch acetylation peaks were observed and their intensities were calculated as 22.56, 23.93, and 22.86 for Acetylated oxidized I, Acetylated oxidized II, and Acetylated oxidized III, respectively. The same amount of acetic anhydride caused the same peak intensity almost. And after Acetylated oxidized II, the area of peaks decreased. The reason for that can be that in the second modification of starch, acetyl groups substituted with the remaining hydroxyl groups in the AGU unit and the increasing amount of oxidant increase the transformation of hydroxyl groups to carbonyl and carboxyl group because of that remaining hydroxyl groups number decreased. These result was also confirmed with DS findings.

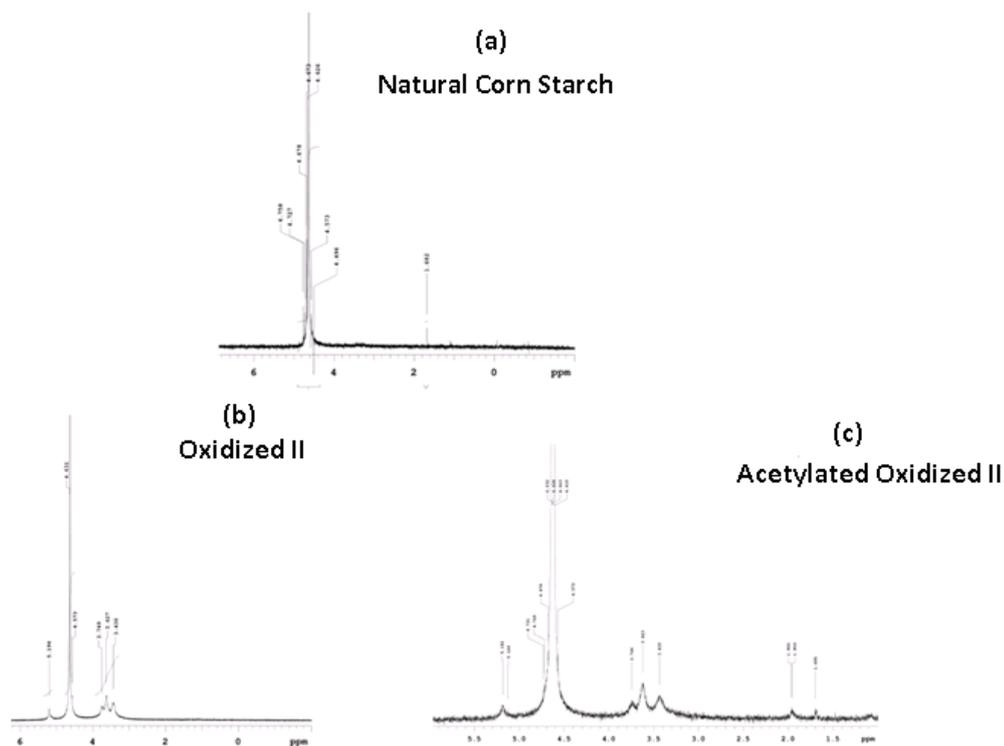


Figure 5.5. H-NMR spectra of (a) natural corn starch, (b) Oxidized II, and Acetylated oxidized II

Oxidized II was selected to study the effect of acetylation agent concentration on modification of oxidized starch due to its carbonyl and carboxyl percentage, solution viscosity and thermal properties.

5.2 Effect of Acetylation Agent Concentration

The effect of acetic anhydride concentration on oxidized starch was investigated with Oxidized II by different acetic anhydride amounts, 7.50, 15.0, and 30.0 g. The results were obtained by titration and H-NMR analysis are given in Table 5.5.

Table 5.5. Acetylation of natural and oxidized II starches with different acetic anhydride amounts

Starch	Active Chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Acetyl Groups (%)	DS by Titration	DS by ¹H-NMR
Natural Corn Starch	-	-	-	-	-
Acetylated	-	15.00	1.475	0.056	0.0059
Acetylated Oxidized IV	12.00	7.50	2.050	0.079	0.0082
Acetylated Oxidized II	12.00	15.00	2.275	0.089	0.0093
Acetylated Oxidized V	12.00	30.00	2.150	0.083	0.0088

As demonstrated in Table 5.5, with the increasing amount of acetic anhydride amount DS increased with acetic anhydride amount of 15 g Acetylated Oxidized II and then decreased . The reason for this might be the number of remaining hydroxyl groups after the oxidation process in starch. This is due to the significant depolymerization of starch molecules and the degradation of crystalline structure caused by oxidation, which allows the oxidant to penetrate deeper into the particles when compared to native corn starch and that oxidized at level VI. The decreased acetylation of starch oxidized at level V compared to level II might well be explained by a reduced number of hydroxyl groups coming from their initial oxidizing to carbonyl and carboxyl groups. And also results showed that double modification DS results (Acetylated oxidized IV, Acetylated oxidized II, and Acetylated oxidized V) were higher than the just acetylated one. This showed that partial depolymerization of starch with oxidation allowed acetic anhydride to penetrate oxidized starch granules more easily thus acetylation occurs more quickly. This result also proved the importance of dual modification. These results were also obtained from the H-NMR analysis with insignificant differences which might be occurred person errors.

FTIR results of natural, oxidized, acetylated, and acetylated oxidized starches were shown in Figure 5.6. As shown in the Figure after acetylation of starch, acetyl groups were formed at wavenumbers of 1735-1740 cm^{-1} , 1368 cm^{-1} , and 1234 cm^{-1} in starch acetate and oxidized starch acetates. The intensity of Acetated Oxidized IV was very

low when compared to the other because of the lower esterification agent concentration. Acetated Oxidized II had higher peak intensity because of its higher DS. Decreasing acetyl groups' peak intensity at higher amount of acetic anhydride, Acetated Oxidized V, showed less acetylation of oxidized starch as determined for its low DS above.

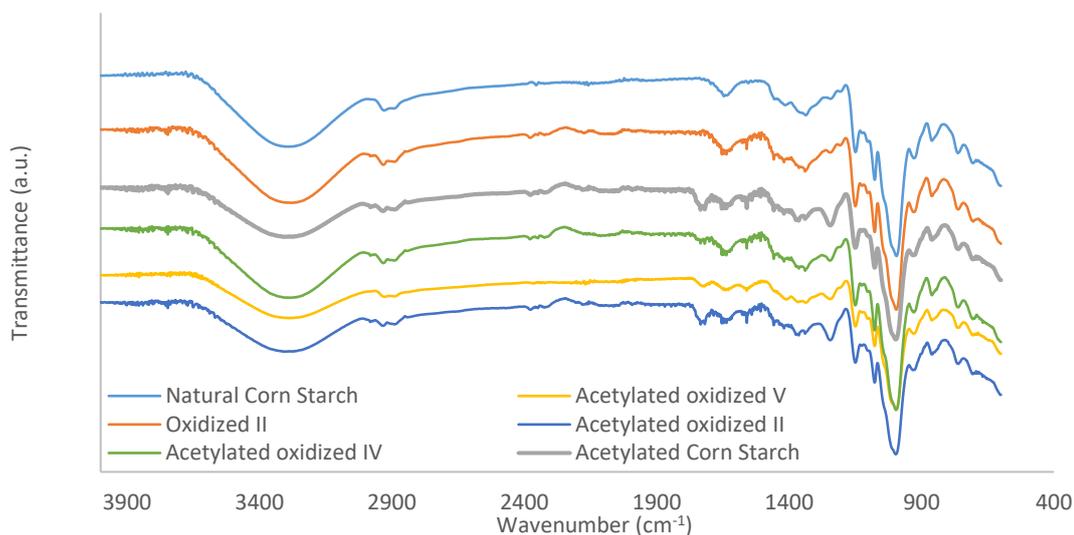


Figure 5.6. FTIR spectra of natural, oxidized, acetylated, and acetylated oxidized starches

The effect of esterification agent concentration on the thermal properties of unmodified acetylated and oxidized acetylated starch was analyzed by DSC. DSC curve of Acetylated Oxidized II was shown in Figure 5.7. As shown in figure T_{onset} , T_{peak} , and T_{end} temperatures were obtained and ΔH value was calculated from the areas of these peaks. Results for other modified starch's were tabulated in Table 5.6.

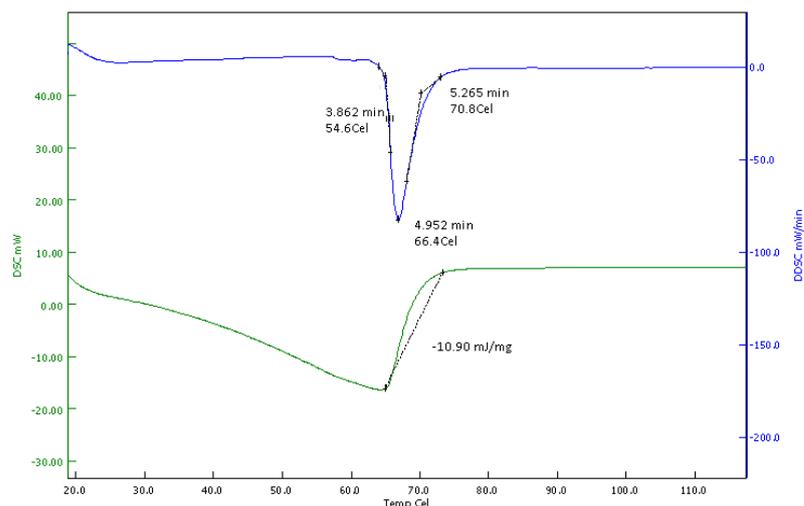


Figure 5.7 DSC and DDSC curve of Acetylated Oxidized II

Natural corn starch had endothermic peaks between 64 and 73 °C, whereas single and double modified acetylated starches had peaks between 52 and 71 °C. When compared to the modified ones, corn starch had a higher gelatinization enthalpy. The acetylation decreased the gelatinization enthalpy until Acetylated oxidized II after this value ΔH value was remained almost constant.

Table 5.6. Thermal properties of modified starch with different acetic anhydrite amount

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	T _{onset} (°C)	T _{peak} (°C)	T _{end} (°C)	ΔT (°C)	ΔH (J/g)
Natural Corn Starch	-	-	64.70	68.40	72.50	7.80	39.40
Oxidized II	12.00	-	67.40	72.70	82.80	15.40	24.00
Acetylated	-	15.00	52.80	59.00	65.20	12.40	23.50
Acetylated oxidized IV	12.00	7.50	63.30	69.80	74.10	10.80	15.80
Acetylated oxidized II	12.00	15.00	54.60	66.40	70.80	16.20	10.90
Acetylated oxidized V	12.00	30.00	65.00	66.90	70.20	5.20	14.30

The addition of acetyl groups to polymer chains caused granular organization instability, resulting in increased swelling and decreased gelatinization temperature. And also, this might be related to the existence of hydrophilic substitution groups in starch granules and an enhancement in hydrogen bonding, which favored gelatinization at low temperatures. Nevertheless, single oxidation and acetylation caused no noticeable reduction in gelling temperature and enthalpy. When comparing the Oxidized II, Acetylated and Acetylated oxidized II, the requirement of double modification was noticed. The decrease in gelatinization temperature after double modification might be related to the loosening of the biopolymer configuration with oxidation firstly and introduction of the bulky groups with acetylation, as well as the concurrent structural reorganization that consequences in a weakening of intramolecular and intermolecular bonding within the starch granules. Thus, minimal energy might be needed for gelatinization. This reduction is important for the industry to the reduction of energy usage.

The light transmittance of natural and modified starch was observed with UV-VIS-NIR Spectra. As demonstrated in Table 5.7 natural corn starch solution had high opacity. With the single and double modifications opacity was diminished. This change was evidence of the destruction of the molecular structure of the starch granule.

Table 5.7. Light transmittance of modified starch with different acetylation agent amount

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Light Transmittance (%)
Natural Corn Starch	-	-	0.03
Oxidized II	12.00	-	8.43
Acetylated	-	15.00	10.32
Acetylated oxidized IV	12.00	7.50	28.96
Acetylated oxidized II	12.00	15.00	37.85
Acetylated oxidized V	12.00	30.00	39.46

The acetylated starches with higher DS values had a lighter transmittance. The reason for that could be that the presence of acetyl groups in the starch granules

allowed the water molecules thus this increased the percent light transmittance and, as a result, the clarity of starch pastes.

The effect of acetylation agent concentration on morphology of oxidized and acetylated oxidized starch are shown in Figure 5.8. Natural corn starch had an angled, big, and smooth surface typical of the corn starch. After oxidation, this shape became a less smooth surface. Some scratches, small fractures, and folds were observed. The acetylated starches changed somewhat in size and shape from their native. After this process, the shape of starch became more scratched and fractured, and some folds were observed and these changes increased with the acetic anhydride concentration. The intermolecular hydrogen bonds were destroyed as the acetyl groups grew, and more granules were ruptured. Acetylated oxidized V showed the highest ruptured. Also, this process caused particle agglomeration. The addition of hydrophilic groups to the starch, which led to a rise in hydrogen bonding, might be related to the fusing of starch granules following acetylation.

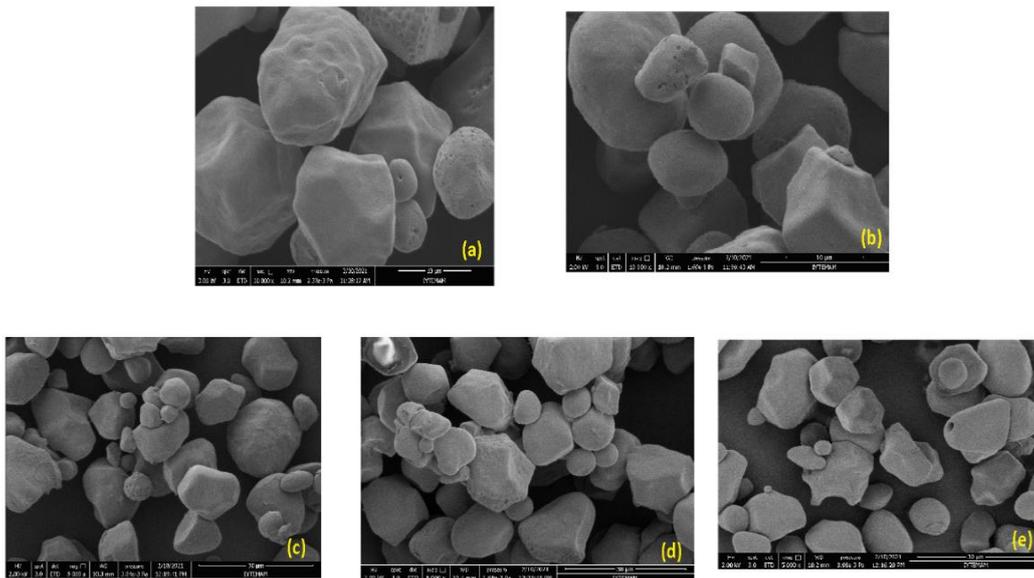


Figure 5.8. Scanning electron micrographs of natural and modified starches with different esterification agent ratios. Native starch (a), Oxidized II (b), Acetylated oxidized VI (c), Acetylated oxidized II (d), and Acetylated oxidized V (e)

Solution viscosity was another important parameter for the product. Table 5.8 shows the solution viscosity of unmodified and modified starches. The measured viscosity values, which are tightly connected with the polymer's molecular mass, allowed analyzing the influence of modification type on starch polymer breakdown. As shown in the table single oxidized starch had a much lower viscosity than single acetylated one. The reason for that could be the depolymerization of starch in the oxidation process. After the acetylation of natural starch, there was lower change in viscosity compared to oxidation. This was probably due to the influence of acetyl groups from neighboring starch granules, which might lead to a rise in their molecular mass. But decrease in viscosity after acetylation might be explained by the fact that acetyl group substitution on starch molecules disrupts intermolecular and intramolecular force interactions by substituting the -OH groups on the AGU, restricting the formation of viscous fluid solutions when compared to natural starch.

Table 5.8. Solution viscosity of natural and modified corn starches

Starch	Active chlorine (g Cl/ kg starch)	Acetic Anhydride (g)	Solution Viscosity (cP)
Natural Corn Starch	-	-	218
Oxidized II	12.00	-	126
Acetylated	-	15.00	168
Acetylated Oxidized IV	12.00	7.50	102
Acetylated Oxidized II	12.00	15.00	96
Acetylated Oxidized V	12.00	30.00	94

Figure 5.9 shows the $^1\text{H-NMR}$ results of acetylated oxidized starch with different concentrations of acetic anhydride. ^1H shifts at 5.10 ppm from oxidation and ^1H shifts at 1.90-2.10 ppm from acetylation were observed. Oxidation peak intensity that was described by 5.10 ppm was calculated as 2.37, 2.40, and 2.27 for Acetylated oxidized IV, Acetylated oxidized II, and Acetylated oxidized V, respectively. It was an expected result because of the same usage dosage of oxidant. The insignificant little changes could be random bonding of carbonyl and carboxyl groups. After the acetylation process of oxidized starch methyl protons were observed at 1.90-2.10 ppm and this peak's intensity changed with the acetic acid concentration. The peak intensities were

calculated as 22.65, 23.93, and 23.62 for Acetylated oxidized IV, Acetylated oxidized II, and Acetylated oxidized V, respectively. Higher peak intensity was obtained in Acetylated oxidized II. These results confirmed the degree of substitution determined by titration.

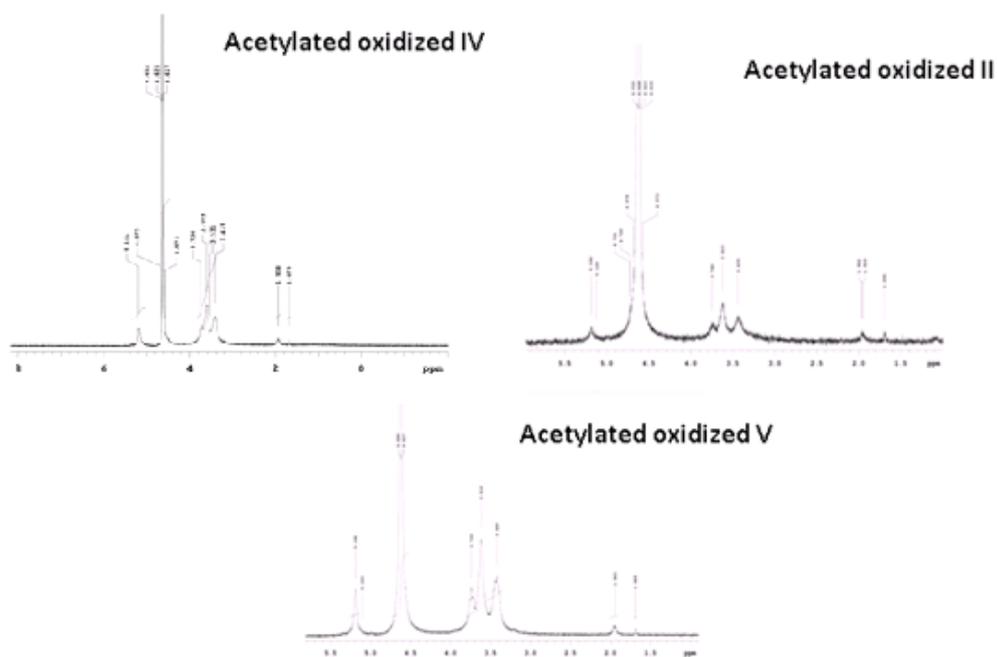


Figure 5.9. ¹H-NMR spectra of acetylated oxidized corn starches with different esterification agent concentration

To sum up, Acetylated oxidized II had the best result according to its solution viscosity, effectiveness and thermal properties for the industry. It had optimum solution viscosity for coating, great effectiveness values to produce adhesive, and lower energy consumption for industrial production.

CHAPTER 6

CONCLUSION

The following conclusions were achieved from the studies performed. Increasing oxidant amount (NaClO) increased carbonyl and carboxyl percentages of all oxidized starches. The highest carbonyl and carboxyl values were obtained as 0.0061% and 1.33% respectively. Acetylation of all the oxidized starches with the same amount acetic anhydride showed that an optimum DS depending of the oxidant amount. Thus the highest DS value (0.089) was obtained for Acetylated Oxidized Starch II. FTIR and ¹HNMR analysis confirmed the findings obtained by titration methods.

Increasing oxidant amount induced enthalpy of gelatinization and increment of light transmittance of natural corn starch in oxidation process. Acetylation of oxidized starches enhanced these properties further. Physical changes were only observed on starch granules with the highest amount of oxidant which was 24 g Cl/ kg starch but acetylation of the oxidized starches created many fractures, scratches, and partial deformations on all oxidized starch. Viscosity of natural starch solution were decreased with increasing oxidant amount from 218 cP to 92 cP due to depolymerization of the starch. However, acetylation modification did not affect viscosity as much as oxidation process. This was attributed to formation of the acetyl group on with each other.

Increasing amount of acetylation for same amount of oxidized starch also gave optimum DS which was considered to be due to depletion of OH groups of oxidized. These behavior was also reflected in thermal property and light transmittance data. Acetylation of starches created more scratched and fractured surfaces with some folds and these changes become more distinguishable with the increasing amount of acetylation agent. Solution viscosity of the oxidized starch were reduced with acetylation but after 15 g. acetic anhydride reduction did not observed markedly.

With the dual modification, natural corn starch was transformed into a material for suitable for adhesive industry. It provided requirement of carbonyl %, carboxyl %, and

DS value, thermal properties, solution viscosity and light transmittance %. According to the findings of the research, Acetylated oxidized II was the best product with its effectiveness values, solution viscosity, and thermal properties.

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