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Enzyme-catalyzed synthesis of hydrophobically modified starch

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Abstract

The reaction of starch and alkyl or alkenyl ketene dimer and starch gave a novel type of hydrophobically modified starch. The reaction was facilitated with the use of an enzyme, which gave products with higher degrees of substitution (DS) at lower temperatures. The starch used included maltodextrin, amylose, cationic starch, and pre-gelatinised starch, with molecular weights ranging from several hundreds to several millions. The DS of the products could be controlled by varying the reaction conditions, such as the amount of enzyme used, the reaction temperature, the concentration of the reactants, and the reaction solvent. This hydrophobically modified starch had improved properties as compared to the original starch and may be used as a thickener or an emulsifier.

Keywords: Aalkyl ketene dimer; Enzyme; Hydrophobic modification; Lipase; Starch

1. Introduction

Starch is a well-known material for industrial use (BeMiller, 1993). In order to improve its end-use properties, starch is sometimes modified with the addition of a hydrophobic functionality. In the past, hydrophobically modified starches are usually prepared by the reaction of starch with fatty acid chlorides (Bader, 1998; Murakami et al., 1989) or fatty acid anhydrides (Billmers & Mackewicz, 1997; Fuertes, Delmotte, & Dreux, 1998; Maliczyszyn, Atkinson, & Tolchinsky, 2000; Narayan, Bloembergen, & Lathia, 1999). Owing to their interesting properties, new types of hydrophobically modified starches are of fundamental interest and may have industrial applications as well.

Alkyl and alkenyl ketene dimers (AKD, Fig. 1) are well-known sizing agents for paper (Brander & Thorn, 1997; Reynolds, 1989). They are produced from either saturated fatty acids (e.g., Aquapel® AKD, Hercules Incorporated) or unsaturated fatty acids (e.g., Precis® ALKD, Hercules Incorporated). They all have a reactive β-lactone functionality that can react with hydroxy- or amino-groups under mild reaction conditions. When used as sizing agents, AKD becomes chemically bound to the cellulose fiber with hydrophobic chains pointing outward, producing a water-repellent surface (Bottorff, 1994; Bottorff & Sullivan, 1993; Cheng & Gu, 2003; Lahteenmaki, Kahkonen, Kloow, & Ruppert, 1999; Nahm, 1986). AKDs are inexpensive materials that produce relatively few by-products after reactions.

Although starch and AKD are readily available, their conjugates are rare (Qiao, Gu, & Cheng, 2002). It was envisioned that AKD could react with starch to make hydrophobically modified starch, and the reaction could be catalyzed with enzymes. Enzymes have been widely used in the synthesis of complex organic molecules (Drauz & Waldmann, 1995; Gijsen, Qiao, Fitz, & Wong, 1996; Koeller & Wong, 2001), and they also have found applications in polymerization and polymer modification reactions (Cheng & Gross, 2005; Gross & Cheng, 2002). The class of enzymes most widely applied to organic synthesis is the hydrolases. Some typical hydrolases are lipases, esterases, and proteases. In this work, we describe the enzyme-catalyzed preparation of AKD-grafted starch,

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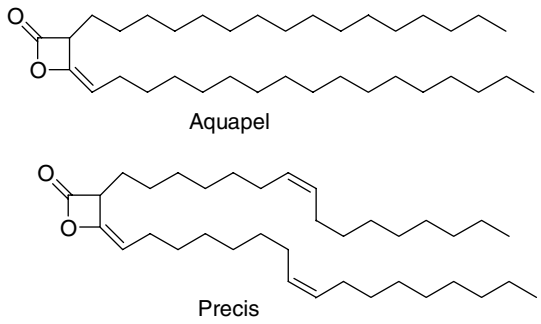


Fig. 1. Structures of AKD.

and also the properties of the products with respect to their degree of substitution (DS).

2. Results and discussions

Lipases, such as porcine pancreatic lipase (PPL), CCL and PS Lipases, are inexpensive and stable enzymes that can catalyze chemo-, regio-, and enantioselective acylation–deacylation of a wide range of substrates (Drauz & Waldmann, 1995). Similarly, proteases are known in some cases to hydrolyze esters (Cheng & Gross, 2005). In this work, we found several selected lipases or proteases that could catalyze the reaction of AKD with starch. Among the enzymes tested, the lipases from *Pseudomonas sp*. and *Pseudomonas fluorescens* gave excellent results. As illustrated in Scheme 1, lipases could catalyze the AKD starch reaction by first forming a covalently linked, highly reactive enzyme-AKD intermediate, which could then react with the hydroxyl group of the anhydroglucose units of starch to form an alkyl β-keto ester.

2.1. Enzyme-catalyzed reactions of AKD and alcohols

We first examine the AKD reaction with simple alcohols. In a typical experiment AKD was dissolved in a solvent, such as *t*-butyl methyl ether. An alcohol and a lipase were then added. The mixture was stirred and incubated at 50 °C for a certain length of time. The product was then isolated by flash chromatography and identified via ^{1}H and ^{13}C NMR. For a number of enzymes, the progress of the reaction is shown in Table 1.

Among the enzymes studied, *P. fluorescens* lipase was the most efficient. Moderate reaction rates were observed for both lipase from *Porcine pancreas* (PPL) and immobilized lipase from *Candida antarctica* (Novozym® 435 lipase from Novozymes A/S). It is worth noting that the reactions with both PPL and Novozym 435 stop after approximately 50% of AKD is converted to methyl 2,4-dialkyl-acetoacetate. These results suggest that both enzymes catalyze the enantioselective alcoholysis of AKD (Scheme 2). The enantiomeric enrichment of AKD was confirmed by optical rotation measurement and ^{1}H NMR analysis in the presence of a chiral shift reagent. However, the alkyl β-ketone ester product was quickly racemized due to enol formation.

Table 1
Estimated conversion of the lipase-catalyzed alcoholysis of AKD

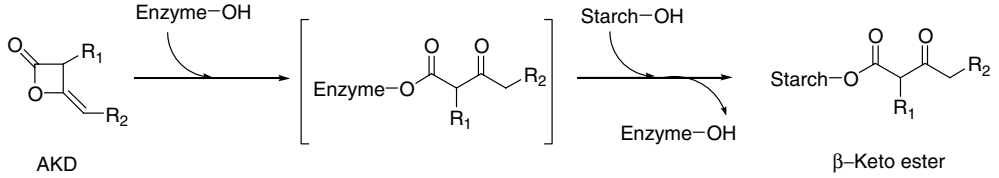
Lipase[a]	Conversion (%) 24 h	Conversion (%) 48 h	Conversion (%) 72 h
Candida antarctica	10	40	40–50
Candida rugosa	<2	<2	<10
Candida cylindracea	<2	<2	<10
Pseudomonas fluorescens	20	75	90
Porcine pancreas	10	45	45–50
Rhizopus delemar	<2	<2	<2
Rhizopus Niveus	<2	<2	<2
None	<2	<2	<2

[a] Lipase from *Candida antarctica* was obtained from Novozymes A/S; Lipase from *Porcine pancreas* was purchased from Sigma–Aldrich. The rest were obtained from Amano Enzyme Inc. Reaction conditions are given in Section 3.1.

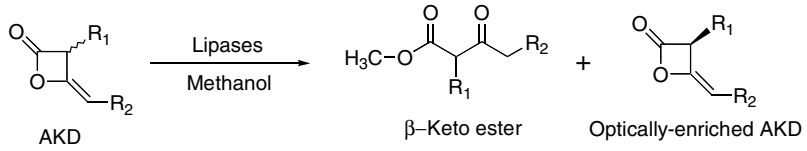
A question may be asked of possible enzyme deactivation in *t*-butyl methyl ether during the reaction. Some loss of activity is unavoidable. In the above reactions, it is important to use a surplus of enzymes to ensure that enough active enzymes were around during the course of the reaction.

2.2. Enzyme-catalyzed reactions of AKD and starch

AKD-grafted starch was prepared in a similar manner. The starting materials include native starches, such as amylose, and modified starches, such as cationic starches and pre-gelatinised starches. The molecular weights of the starches range from several hundreds to several millions. In a typical experiment, starch was dispersed in a solvent, such as dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), or *N*,*N*-dimethylacetamide (DMAc). AKD was added to the solution followed by an enzyme. The reaction was then incubated at 50 °C. The product was isolated by precipitation with isopropyl alcohol or acetone. The unreacted and hydrolyzed AKD was removed by washing or by Soxhlet extraction with organic solvents. Solvents that could be used included methylene chloride,



Scheme 1. Enzyme-catalyzed formation of starch alkyl β-ketone ester.



Scheme 2. Enantiomeric alcoholysis of AKD.

chloroform, hexane, ethyl acetate, and acetone. The degree of substitution (DS) was calculated from the ^{1}H NMR spectrum (Fig. 2).

The effect of enzyme amount on the reaction was studied. As illustrated in Fig. 3, at 50 °C, AKD reacted with starch in DMSO to give the AKD-grafted product with low DS values. In the presence of lipase PS at 8 weight % relative to starch, the reaction proceeded much faster and yielded products with higher DS values. As the amount of enzyme further increased (to 24% relative to starch), the reaction proceeded more efficiently. However, it was also found that the DS of the product did not increase after 4 h. This was likely due to the hydrolysis of AKD in DMSO and possibly also to partial deactivation of the enzyme. It may be noted that homogeneous solutions were obtained in DMSO for all the starches. With DMF or DMAc, suspensions were found.

In addition to enzyme concentration, other reaction parameters were also used to regulate the DS of the AKD-starch adduct, e.g., the concentration of AKD, the reaction time, the solvent used, reaction temperature, and pH. In general, higher AKD concentration, longer reaction time, higher temperature, and higher pH tended to give products with higher DS values.

2.3. Non-enzymatic reaction

AKD also reacted with starch in the absence of an enzyme; however, the efficiency was not as high as the enzyme-catalyzed reactions. In order to achieve higher DS products, we need to employ higher reaction temperatures and/or use a base.

In the non-enzymatic reaction, the reaction is typically carried out at about 50–120 °C for 0.5–24 h. Although the presence of organic solvent mediums helps the reaction to be carried out more efficiently, the reaction can also proceed without the use of any solvent. This reaction is facilitated via the use of a base, both inorganic and organic. Typically, sodium hydroxide was employed as the inorganic base, and 4,4-dimethylaminopyridine (DMAP) as the organic base. The non-enzymatic reaction without a base proceeds at a much slower rate.

At the end of the reaction, the reaction is neutralized with an acid and the product is precipitated by an organic

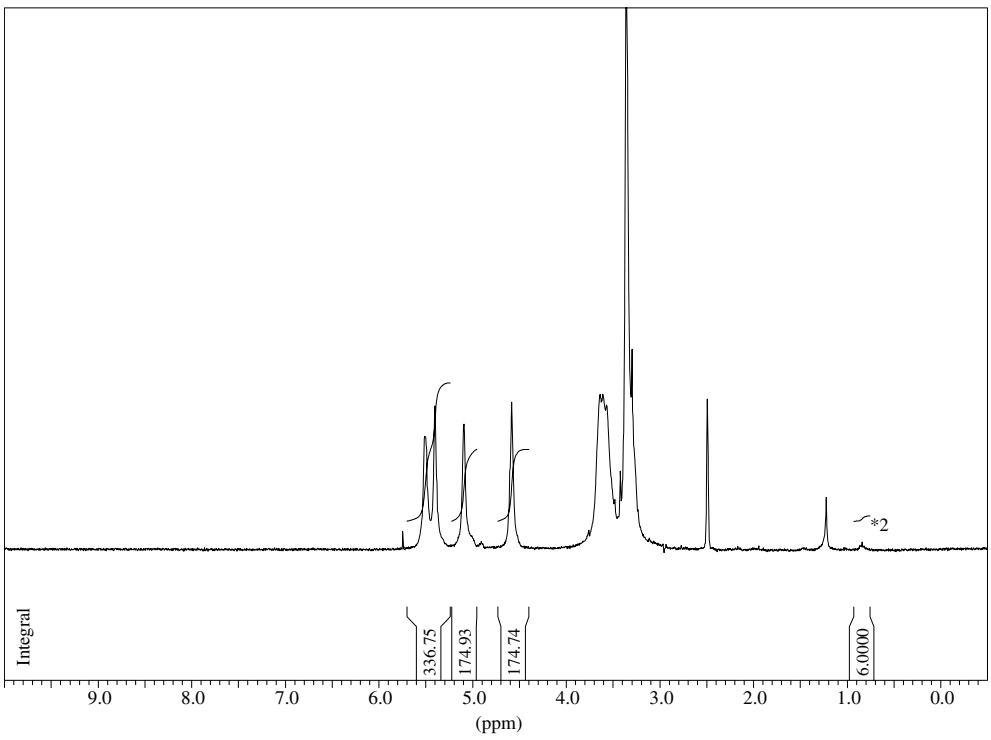


Fig. 2. ^{1}H NMR spectrum of the starch–AKD adduct. Resonances at ca. 1.18 and 0.80 ppm correspond to CH_2 and CH_3 of AKD respectively and can be used to estimate DS.

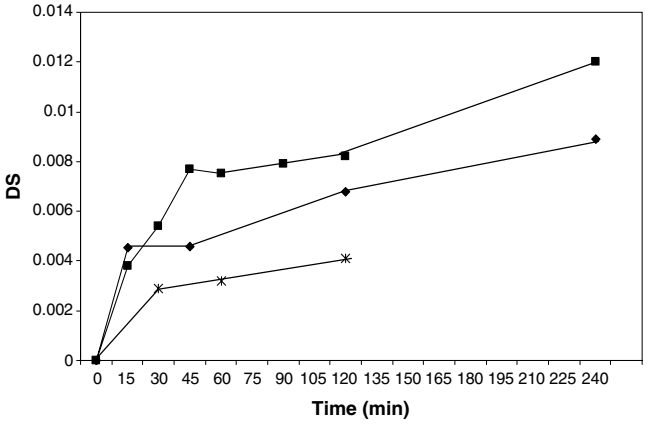


Fig. 3. The effects of enzyme amount on the DS of the starch–AKD adduct. Lower curve, no enzyme; middle curve, 8% enzyme; top curve, 24% enzyme.

solvent, such as isopropyl alcohol, acetone, or other similar organic solvents. The precipitated product is then washed with at least one of methylene chloride, chloroform, hexane, ethyl acetate, acetone or other similar organic solvents. Soxhlet extraction can be performed on the washed product with acetone, ethyl acetate, hexane, chloroform, methylene chloride or other similar organic solvents. The product is then dried under vacuum.

2.4. Comparison of chemical vs. enzymatic synthesis

Although the reaction between starch and AKD can be carried out via either enzymatic or chemical methods, the two methods are complementary, and there are relative advantages and disadvantages. Some of the differences are summarized in Table 2. Obviously, the enzymatic reaction can be carried out under milder reaction conditions with less AKD. Furthermore, in the chemical method, the use of acid or base and higher temperature can cause degradation of the starch and result in products with much lower viscosity. This is an advantage for the enzyme method. On the other hand, the enzyme adds to the raw material costs and the cost/benefit ratio needs to be considered in a commercial setting.

2.5. Properties of starch–AKD adducts

As AKD is derived from two fatty acid residues, two hydrocarbon chains are grafted onto starch simultaneously,

Table 2
Comparison of enzymatic and chemical synthesis

Enzyme method	Chemical method
10 wt% AKD used	Excess of AKD used
40–50 °C temperature	80–120 °C temperature
No acid or base	NaOH addition helps
No degradation of starch	Starch partly degraded
Product viscosity high	Product viscosity lower

Table 3
Effect of reactant concentrations on the DS and the solution viscosity of the product

Weight of starch (g)	Weight of AKD (g)	Weight of lipase PS (g)	DS	Viscosity (cps)
20	0	0	0	546
20	0.8	1.6	0.0032	1256
20	1.6	1.6	0.0042	1648
20	3.2	2.4	0.0046	1948
20	3.2	1.6	0.0052	912

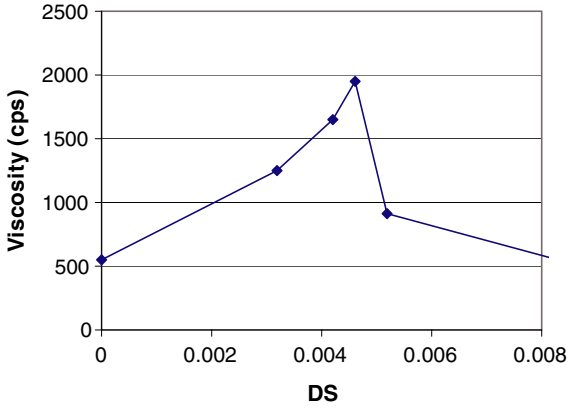


Fig. 4. Brookfield viscosity vs. DS for the AKD-starch adduct.

rendering the product highly hydrophobic. Thus, a starch modified with AKD has higher Brookfield viscosity and lower water solubility compared with the original starch. As the DS increases, the solubility of the product decreases; on the other hand, the viscosity of the product solution increased initially and decreased as the product became less water-soluble. This is illustrated in Table 3 and Fig. 4.

The increased viscosity of the starch–AKD adducts versus the starting starch suggests that this material is a good thickener. The viscosity of the solution remained substantially unchanged for at least three days when the solution was stored at pH of about 6.5–8.5. The data also indicate the presence of a window of opportunity. In order to produce an optimal thickener, the DS is preferably in the range of 0.003–0.005. The starch–AKD adducts at lower DS values have lower viscosities and perhaps can be used as emulsifiers and polymeric surfactants.

3. Experimental

3.1. Preparation of AKD–alcohol adducts

AKD (Aquapel® 36H, Hercules, 0.2 g) was dissolved in 4 mL *t*-butyl methyl ether containing 0.2 mL methanol and 0.05 g lipase P (from *P. fluorescens*, Amano). The mixture was stirred at 50 °C for 48 h. TLC analysis showed a new spot at R_f 0.3 (SiO_2, EtOAc/Hex, 1:9, detected by I_2 vapor or H_2SO_4. R_f of AKD = 0.45). The product was isolated by flash chromatography (silica, EtOAc:Hex = 10:1) and identified to be methyl 2,4-dialkyl-acetoacetate: ^{1}H NMR ($CDCl_3$, 300 Hz) 3.73(s, 3H, CH_3O–), 3.59–3.34(t, 1H,

CH-COOR), 2.50–2.20 (m, 2H, –$COCH_2$–), 1.76(m, 2H, CH_2–C–COO–), 1.18(m, 60H, –CH_2–), 0.81–0.78(t, 6H, –CH_3); $^{13}C(CDCl_3$, 75.5 Hz) 205.5(–CO–), 170.4(–COOR), 51.4(CH_3O–)].

3.2. Enzyme-catalyzed reaction of AKD and starch

3.2.1. General procedure

The starch sample was dissolved or suspended in a solvent. Slight heating might be required to expedite this process. AKD (powdered Aquapel® or Precis®) was added followed by the addition of an enzyme. The reaction mixture was incubated at the temperature and for the length of time required. The reaction mixture was then poured into acetone or isopropyl alcohol with rapid stirring. The precipitates were collected by vacuum filtration. The solid was purified by Soxhlet extraction with a solvent (acetone, ethyl acetate, hexane, chloroform, or methylene chloride), or alternatively washed with chloroform or methylene chloride for 3–4 times. The solid was then dried at 50 °C under vacuum to consistent weight and characterized by ^{1}H NMR and IR. Viscosity and solubility were measured in water.

3.2.2. Specific procedure for AKD–amylose

Amylose (5.0 g) and Aquapel® 364 (from Hercules, 0.8 g) were suspended in DMAc (100 mL). Lipase PS (from Amano, 0.4 g) was added, and the reaction mixture was incubated at 50 °C. The reaction mixture was then poured into isopropyl alcohol. The precipitate was washed successively with CH_2Cl_2 and hexane. The solid was then dried at 50 °C under vacuum to consistent weight. ^{1}H NMR (DMSO-d_6) δ 5.6 (s), 5.4 (s), 5.1 (s), 4.55 (s), 3.80–3.30 (m) 1.45 (m), 1.25 (s), 0.8 (t). The DS was determined to be 0.0057 by ^{1}H NMR.

3.3. Non-enzymatic reaction of AKD with starch

3.3.1. General procedure using a solvent

A mixture of cationic starch 52A (from National Starch, 2.5 g), Aquapel® AKD (from Hercules, 8.2 g), and NaOH (0.6 g) in DMSO (100 mL) was heated at 110–120 °C under an atmosphere of N_2 for 5 h. The reaction mixture was cooled to room temperature and poured into a mixture of acetone containing acetic acid (200 mL: 0.93 mL). The precipitate was then washed three times with $CHCl_3$ and dried under vacuum to give a yellowish solid (1.9 g). The DS of the material was determined to be 0.016.

3.3.2. General procedure without the use of a solvent

Aquapel® 364 AKD (0.8 g) was dissolved in CH_2Cl_2 (10 mL). The solution was added to cationic starch 52A (from National Starch, 5.0 g). The CH_2Cl_2 was then removed with a rotary evaporator. The resulting solid was heated at 85–90 °C for 24 h and then washed with chloroform 3–4 times and dried under vacuum to give a white powder. The degree of substitution of the product was determined by ^{1}H NMR to be 0.0025.

3.4. Brookfield viscosity measurements

The viscosity (in centipoise) was measured at 24 °C using a Brookfield DV-1 viscometer, with number 2 spindle at a speed of 30 rpm. A suspension of 4 wt % of starch sample in distilled water was heated at 90 °C for 30 min and then cooled to room temperature for measurement.

4. Conclusions

In this work a new type of hydrophobically modified starch was prepared by enzyme-catalyzed reaction of starch and AKD. The reactions without an enzyme proceeded less efficiently and could be facilitated by the addition of a base or heating at higher temperatures. The properties of the AKD–starch adducts depend on the degree of substitution. If the product is to be used as a viscosity builder in aqueous solutions, we need to target a DS between 0.003 and 0.005, or thereabouts. This new material may perhaps be used as an ingredient in applications where high solution viscosity and hydrophobic interactions are desired. Some examples are paint thickener, construction material, emulsion stabilizer, and emulsifier for personal care products.

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References

Bader, H. (1998). *Process for the manufacture of biodegradable materials.* Patent Publication EP0859012 A2.

BeMiller, J. N. (1993). Starch-based gums. In R. L. Whistler & J. N. BeMiller (Eds.), *Industrial gums* (3rd ed., pp. 579–600). Sa Diego: Academic Press.

Billmers, R. L., & Mackewicz, L. (1997). *Process for preparation of hydrophobic starch derivatives*. Patent Publication US 5,672,699.

Bottorff, K. J. (1994). AKD sizing mechanism: A more definitive description. *Tappi Journal, 77*, 105.

Bottorff, K. J., & Sullivan, M. J. (1993). New insights into the AKD sizing mechanism. *Nordic Pulp and Paper Research Journal, 8*, 86.

Brander, J., & Thorn, I. (1997). *Surface application of paper chemicals.* London: Blackie Academic.

Cheng, H. N., & Gross, R. A. (2005). In *Polymer biocatalysis and biomaterials. ACS Symposium Series 900*. Oxford: Oxford University Press.

Cheng, H. N., & Gu, Q. -M. (2003). *Esterified polysaccharide products with ketene dimer by beta-lactone ring opening*. Patent Publication US 6,528,643.

Drauz, K., & Waldmann, H. (1995). *Enzyme catalysis in organic synthesis: A comprehensive handbook*. Weinhiem: Wiley-VCH.

Fuertes, P., Delmotte, L., & Dreux, J. -L. (1998). *Cationic polysaccharides esterified by a discarboxylic acid anhydride substituted with a branched carbon chain*. Patent Publication US 5,731,430.

Gijsen, H. J. M., Qiao, L., Fitz, W., & Wong, C.-H. (1996). Recent advances in the chemoenzymic synthesis of carbohydrates and carbohydrate mimetics. *Chemical Reviews, 96*, 443–473.

Gross, R. A., & Cheng, H. N. (2002). In *Biocatalysis in polymer science. ACS Symposium Series 840*. Oxford: Oxford University Press.

Hercules Incorporated. (2000). A brochures on Aquapel® and Precis® sizing agents are available at <http://ppd.herc.com/literature/files/sizing_printability_technology_brochure.pdf>.

Koeller, K. M., & Wong, C.-H. (2001). Enzymes for chemical synthesis. *Nature, 409*, 232–240.

Lahteenmaki, M., Kahkonen, H., Kloow, G., & Ruppert, O. (1999) *Method for the preparation of modified cellulose ethers*. Patent Publication WO 99/61478.

Maliczyszyn, W., Atkinson, J. W., & Tolchinsky, M. (2000). *Method for preparing hydrophobic starch derivatives*. Patent Publication US 6,037,466.

Murakami, K., et al. (1989). *Production of water-soluble starch fatty acid ester*. Patent Publication JP 1,054,001 A2.

Nahm, S. H. (1986). Direct evidence for covalent bonding between ketene dimer sizing agents and cellulose. *Journal of Wood Chemistry and Technology, 6*, 89.

Narayan, R., Bloembergen, S., & Lathia, A. (1999). *Method of preparing biodegradable modified starch moldable products and films*. Patent Publication US 5,869,647.

Qiao, L., Gu, Q. -M., & Cheng, H. N. (2002). *Hydrophobically esterified starch products and processes of making the same.* Patent Application US 20020123624A1.

Reynolds, W. F. (1989). *The sizing of paper* (2nd ed.). Atlanta: TAPPI.