MULTIFUNCTIONAL STARCH DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND PROPERTIES

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MULTIFUNCTIONAL STARCH DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND PROPERTIES

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Abstract

Amongst polysaccharides, starch is one of the most widely produced biopolymer in the world. Because of biocompatibility and degradability, the biopolymer has been of interest in pharmaceuticals, thermoplastics, paper, textile and several other applications. Generally, starch is modified chemically, physically or enzymatically to increase its usefulness and to fulfill the various demands for functionality of different starch products. An area of growing interest is the production of multifunctional starch derivatives for applications such as biodegradable delivery systems. In the research described in this thesis a two step approach was chosen in order to develop and optimize an environmentally friendly process for the synthesis of novel, highly reactive granular epoxy starch derivatives.

Via this mild method, maize starch was substituted with allyl glycidyl ether (AGE) followed by epoxidation of the double bond. Granular unsaturated maize starch derivatives were synthesized by alkali-mediated etherification of maize starch with AGE, resulting in a degree of substitution of 0.20. It was shown by NMR that the glucose moiety of the starch is mainly substituted at the O-6 position. At polymer level, the allyl groups are clustered and randomly distributed along the polymer chain, depending on the type of starch. Experimental design approach was used to optimize process conditions on the synthesis of different maize starches with AGE. Statistical analysis of the results showed significant impact of the temperature, the NaOH concentration and the amount of AGE on the modification of maize starch. In the optimization process, different optimal conditions were found for the reaction of different maize starches with AGE. Rate and efficiency of starch substitution depends on the type of maize starch and reaction conditions. Incorporation of AGE affected the behavior and properties of the maize starches. This etherification leads to increased swelling and solubility of the maize starch granules, decreased gelatinization, altered pasting properties and flow behavior. Eventually, the double bonds in allyl starch were epoxidized. Enzyme-resistant oligomers of epoxy starch derivatives elucidated that a large amount of allyl groups was converted into epoxy groups. However, only a small amount of epoxy groups could be detected by a spectrophotometric method. It is postulated that the inherent reactivity of epoxy groups may have led to subsequent side reactions. In conclusion, epoxy starch derivatives were conveniently synthesized through the presented method. Further experiments are required in order to assess the applicability of these epoxy starch-based products.

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Chapter 1

General introduction

1.1 Structure and properties of starch

1.1.1 Starch

After cellulose, starch is the most abundant carbohydrate available from agricultural raw material. The estimated world production of starch amounts to 58 million tonnes, extracted from maize (46 million), wheat (4.6 million), potatoes (3.5 million), and the remainder coming from rice and cassava roots (tapioca).[1] Starch is the main carbohydrate in plants and acts as a reserve food supply for periods of growth, dormancy and germination.[2-5] Being a biodegradable polymer with well-defined chemical properties, it has a huge potential as a versatile renewable resource for various material applications in food and nonfood areas. The composition and properties of commercial available starches have been studied extensively.[2,3,5-11] The properties of each starch are strongly dependent on their plant source.

1.1.2 Molecular structure: amylose and amylopectin[2,3,5-11]

Starch is a heterogeneous polymer of α-D-glucose units. The anhydrous glucose units (AGUs) are mainly linked by α-(1,4)-bonds and to some extent by α-(1,6)-linkages. The biopolymer consists of two distinguished structural forms: amylose and amylopectin.

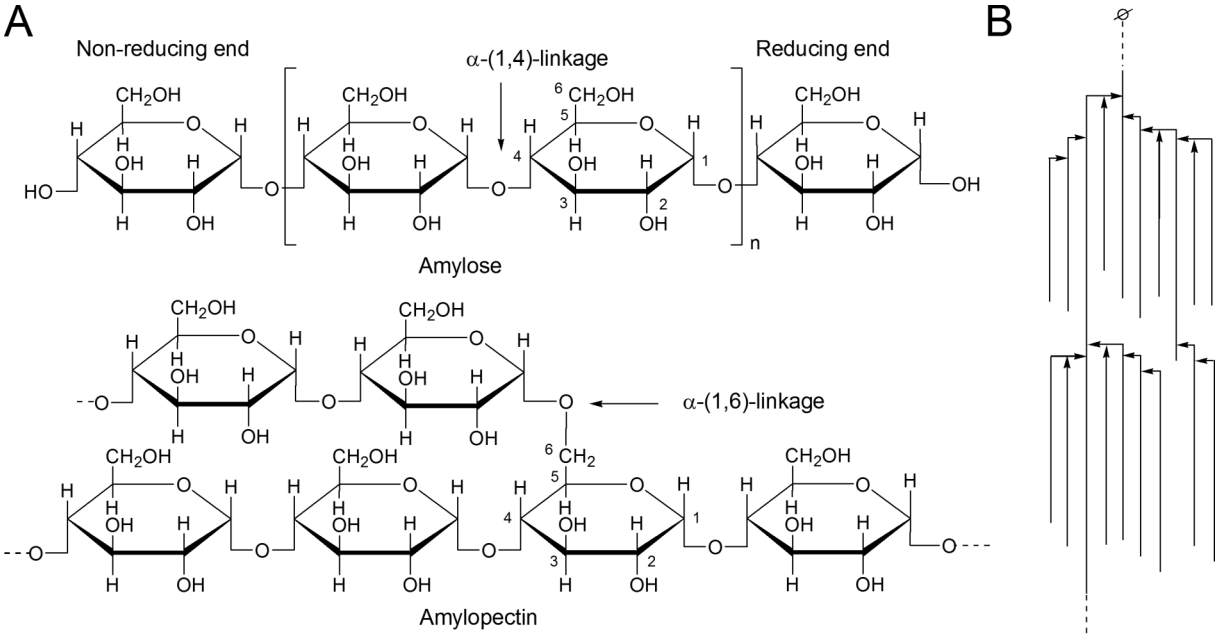


Figure 1.1. (A) Linear chain structure of amylose, and amylopectin structure with a branch point at the α-(1,6)-position. (B) Schematical cluster model of amylopectin; ⌀ is reducing end of starch molecules.

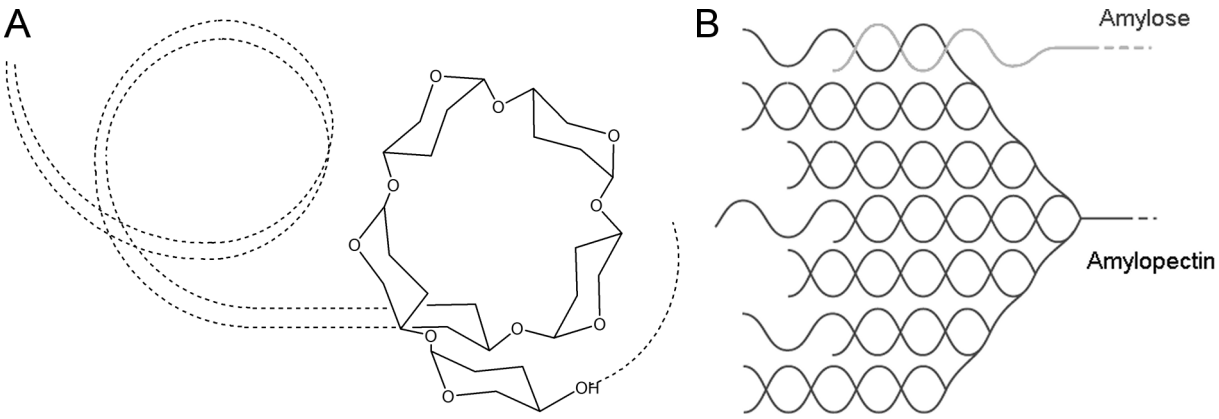


Figure 1.2. (A) Structures of single helix amylose. (B) Schematical cluster model of amylose and amylopectin organized in double helical structures.[12]

Amylose is mainly found as a long linear polymer containing about several hundred α-(1,4)-linked glucose units (up to 6000 AGUs), with a molecular weight of 10^5-10^6 g mol^{-1} (Figure 1.1). In the solid state, the chains very easily form single or double helices (Figure 1.2). In contrast, amylopectin is a highly branched molecule with a molecular weight of 10^7-10^9 g mol^{-1} (Figure 1.1). The branched polymer contains α-(1,4)-linked glucose units but has additional α-(1,6)-glucosidic branching points which are believed to occur every 10 to 60 glucose units, i.e. 5% of the glucose moieties are branched (Figure 1.1). With an average degree of polymerization of about 2 million, this biopolymer is one of the largest molecules in nature. Besides amylose and amylopectin, starch granules contain (very) small amount of protein, lipids and inorganic compounds.

1.1.3 Variety and properties of starch granules

Native starches differ in the amylose/amylopectin ratio depending on their botanic source, e.g. native starches are composed of 20-30% amylose and an additional amount of amylopectin, amylose-enriched starch may contain up to 84% amylose,[13] while waxy starches consist of nearly pure amylopectin. The commercial available starches can be divided in three major groups.[2,9] The first group consists of cereal starches (maize and wheat), whereas the second group is composed of tuber (potato) and roots starches (tapioca and rice). These first two groups are distinctly different from each other with respect to composition and physical properties such as the size of the granule, the gelatinization temperature as well as the type of crystallinity.[9] The waxy cereal starches are the third group and their physical properties are comparable to those of tapioca starch.

Microscopic studies have revealed that starch granules can vary in shape, size and composition depending on the source of starch. In general, the appearance of the starch granules varies from small granules (cereals) to large granules (tuber and root), ranging from 1 to 100 μm. The shape of a granule can be oval, spherical, polygonal or truncated.[9,14]

1.1.4 Crystalline organization of native starches

The native starch granule consists of a semi-crystalline structure. The molecules of amylose and amylopectin are organized and packed as single and double helices (Figure 1.2) into small granules. Amylose and the branching points of amylopectin form the amorphous region. Amylopectin in the granule is the main crystalline structure in the granular starch, consisting of double helices.[8,10,11,15] Depending on the moisture and the source, the degree of crystallinity in starch varies between 15 and 51%.[10,11,13,16,17] For native starch, the type of crystalline patterns are labelled as A-, B- and C-type crystalline pattern.[11] The A-type pattern is characteristic for cereal starches, the B-type for tuber starches. The differences between A- and B-types result from the packing of the double helices in the crystalline structure and the quantity of water molecules stabilized in the unit cell.[10,18] C-type starches (e.g. pea starch) is a mixture of A- and B-type.[10,17-20] After gelatinization of the granular starch, the amylose and amylopectin are able to recrystallize into several structures.[15,21-23] They are able to recrystallize into A-, B- and C-type structures or into several single helical V-type structures of amylose molecules complexed to lipids or fatty acids.[18,22,23] The kinetics of the recrystallization process and the resulting crystalline composition depend on starch source, the moisture content, the process conditions, and additives.[11,20,21,24]

1.1.5 Swelling and solubility

Starch swells when heated in excess water. Water penetrates into the amorphous regions and disrupts the intra- and intermolecular hydrogen bonds in the crystalline area, resulting in granule swelling and solubility.[7,25-27] Amylopectin contributes to the granule swelling, whereas amylose and lipids inhibits the swelling.[9,28] At the gelatinization temperature, the swelling of the amorphous phase accelerates the disruption of the crystalline region. Eventually amylose leaches out of the granule. Factors like the starch source, (e.g. amylose/amylopectin ratio, branching and length of the amylopectin), degree of

granulation and other factors influence the swelling and solubility. Swelling power and solubility index provide evidence of the magnitude of interaction between the starch molecules within the amorphous and crystalline region.[28-30] Swelling power is defined as the weight of sedimented swollen granules to the initial weight of dry starch. The solubility index is expressed as the percentage (by weight) of the starch sample that is dissolved after heating in water.

1.1.6 Dispersion properties

When starch granules are heated in the presence of excess water, granule shape, crystallinity, optical properties such as birefringence are initially retained until a certain temperature. Above this so-called gelatinization temperature, the starch granules swell irreversibly and eventually release amylose into solution.[27,31] Strictly, *gelatinization* of starch is an order-disorder phase transition in which granular swelling, crystalline melting, loss of birefringence, viscosity development and solubilization of amylose occurs.[7,27,28,31,32] The gelatinization temperature is influenced by the amount of water available as well as the presence of other chemicals, and the degree of crystallinity.[7,18,28,30] When a majority of granules have undergone gelatinization, the starch is considered to be "paste".

As heating continues and more and more granules become swollen, the viscosity of the medium increases. A maximum viscosity is reached when the largest percentage of swollen intact granules is present which is referred to as peak viscosity (see rheology). At this point, the starch is considered to be fully pasted. For native starches, continued heating eventually results in a decrease in viscosity as the granules dissolve and the polymers are solubilized. *Pasting* is the phenomenon following gelatinization in the dissolution of starch.[31-33] It involves granular swelling, exudation of the granular compounds and total disruption of the granules.

When the starch suspension is cooled, the starch molecules begin to reassociate in an ordered structure, forming a gel. In dilute solutions, the aggregate chains of amylose will precipitate. The process of alignment, association and precipitation is known as *retrogradation*.[7,31,33] Highly concentrated swollen starch suspensions will form elastic gels or starch pastes exhibiting gel like properties (e.g. viscoelastic and flow behavior) and appearance (e.g. transparancy, texture).[5,9,21] Rheological properties of a starch paste are

highly dependent on the initial gelatinization conditions governed by the starch source, concentration, granule size and the method of observation.[5,32,34-36]
The structural changes during gelatinization have been revealed with several sensitive techniques such as differential scanning calorimetry, small-angle or wide-angle X-ray scattering (SAXS or WAXS),[8,18,37,38] dynamic rheometry,[7,39,40] ^{13}C NMR[41,42] and viscosity measurements using the Brabender viscoamylograph and Rapid Visco-Analyser (RVA).[33,43]

1.1.7 Rheology

The gelatinization and pasting of starch is often followed by measuring the rheological changes in a viscometer. Small changes in starch structure show large changes in viscosity of the starch dispersion upon heating, depending on the shear applied during measurement. Commonly, the viscosity of the starch-water dispersion is measured with a Brabender viscoamylograph, an Ottawa Starch Viscometer or a Rapid Visco-Analyser (RVA).[43-45] The RVA is becoming increasingly popular for the analysis of the starch gelatinization and pasting. The distinct advantages of the RVA are small sample size, ability to set temperature faster, computerized control, data collection and more facile calibration procedures.[43,45,46]

After a short pregelatinization phase, suspensions are stirred and heated at a constant rate, held at any desired temperature and subsequently cooled at a constant rate. Due to the faster and stronger mixing, the RVA leads to both more rapid peak development and more rapid breakdown compared to the other techniques. The typical pasting curve, measured in RVA units (RVU, approximately equal to cP × 12), is continuously plotted against time as shown in Figure 1.3.

The RVA profile includes the following terms: *Pasting (onset) temperature* is the temperature at which a perceptible increase in viscosity occurs. *Peak viscosity* is the maximum viscosity which can be reached when starch is gelatinized upon heating. The temperature and time corresponding to the peak viscosity are referred to as *peak temperature* and *peak time*, respectively. *Holding strength* or *hot paste viscosity* is the minimum viscosity in the phase of high temperature and constant shear stress. The viscosity at this stage is caused by further disruption of the granules, leaching of amylose molecules and alignment of polymers. *Breakdown* or *pasting breakdown* represents the decrease in viscosity between peak viscosity and hot paste. *Final viscosity* is the viscosity

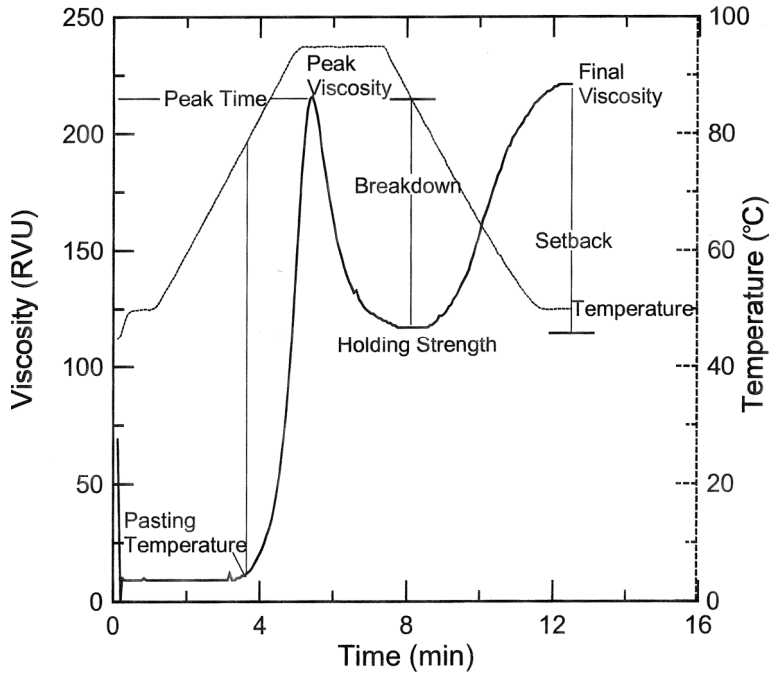


Figure 1.3 Typical Rapid Visco-Analyser pasting curve showing the commonly measured parameters.

after cooling which indicates the ability of the material to form a viscous paste or gel after cooking and cooling. *Setback* refers to change in viscosity during retrogradation and is the phase between the hot paste and final viscosity.

1.2 Modification of starch

1.2.1 Chemical modification of starch

Starch is very often modified to produce derivatives with improved properties for specific applications and industrial use. Commonly, starch is modified by chemical reactions and enzymatic treatment. Also, the starch granule can be modified by physical treatment such as extrusion, gelatinization, blending and drying.[15,25,47,48] Chemical modification is based on reaction of the free hydroxyl groups of the AGU monomers with a functional group, resulting in starch derivatives. Usually, the modification involves esterification or etherification of the hydroxyl groups. In general, these modifications of the hydroxyl groups in starch in the presence of small amounts of base favors O-2 substitution above O-3 and O-6 substitution. The hydroxyl group on C-2 exhibits the highest acidity and is therefore the most reactive. However, the other groups may also react because the reactivity of the hydroxyl groups depends on electronic and conformational factors, and their availability for reagents in general.[47,49]

Table 1.1. Properties and application of the main commercially available starch derivatives.

Modified starch	Reagents	Properties	Application
Acetylated[49-54]	Acetic anhydride Vinyl acetate	High viscosity, decreased gelatinization and retrogradation, improved storage stability, good film properties	*Food*: thickeners, stabilizers; *paper*: surface sizing agents; *textile*: warp sizing agents; gummed tapes
Phosphorylated[49,55-57]	Phosphoric acid	Decreased gelatinization, high viscosity, stable dispersion, cohesive texture, polyelectrolyte	*Food*: emulsifiers, thickening agents, adhesives; *paper*: wet-end additives and binders; *textile*: thickeners, warp sizing agents, stiffening; *pharmaceuticals*: detergents; encapsulation; flocculants
Succinylated[30,49,58-60]	Succinic anhydride Octenyl succinic anhydride	High swelling, viscous pastes, reduced retrogradation, improve freeze thaw stability	*Food*: additives to beverages, binders and thickening agents; *paper*: surface sizing agents, coating binders; films; *pharmaceuticals*: encapsulation, drugs delivery systems, tablets decomposer
Alkylated[61-65]	Propylene oxide Ethylene oxide Acrylonitrile	Decreased gelatinization, lower pasting temperature, reduced retrogradation, improved storage stability, increased swelling, film properties	*Food*: thickening agents, coatings; *paper*: surface sizing, coatings and binders; adhesives for plastic shaping; films; *pharmaceuticals*: drugs and protein carriers
Carboxymethylated[49,66-68]	Monochloroacetic acid	Enhanced solubility, improved clarity, polyelectrolyte	*Paper*: hydrophilic sizing agents, coatings; *textile*: sizing agents; *pharmaceuticals*: anti-tumor, drugs delivery system; chelation ion-exchange polyanion flocculations; mostly cross-linked (absorbent product)
Cationic[49,69-72]	(3-Chloro-2-hydroxypropyl) trimethylammonium chloride (2,3-Epoxypropyl) trimethyl-ammonium chloride	Better dispersity and solubility, polyelectrolyte	*Paper*: wet-end additives and binders, coatings, surface sizing agents; *textile*: warp sizing agents; flocculants; detergents
Cross-linked[73-76]	Phosphorous oxychloride Sodium trimetaphosphate Epichlorohydrine	Decreased gelatinization, reduced swelling and solubility, reinforced granules, improved storage stability	*Food*: thickener agents; *paper*: wet-rub resistant coating and adhesives; emulsions; surgical dusting powder; anti-perspirants, personal sanitary applications

The behavior and properties achieved after chemical modification are depending upon the number, the distribution and the nature of the substituents as well as the starch source and reaction conditions.[14,47] Those starch derivatives with a degree of substitution up to 0.20 are of commercial importance.[5,47-49,77-79] Commercially available starch derivatives display a variety of properties which are suitable for industrial applications (Table 1.1).

Generally, these starch derivatives can be manufactured in an aqueous solution at a temperature below 60°C. NaOH and Na_2SO_4 can be added, respectively, to enhance the reactivity of the hydroxyl groups in starch and to prevent gelatinization of the granular starch under alkaline conditions.[47,78] Under these conditions, the granular structure of starch can be maintained, and subsequently granular starch derivatives can easily be recovered by simple filtration and drying. Starch modification generally leads to a reduction in gelatinization temperature. This implies that only a limited level of substitution can be obtained when one wants to retain the starch in granular form.[78]

The degree of substitution (DS) is defined as the average number of hydroxyl groups on the AGU that have been substituted.[3,78] DS ranges from 0 to 3. If DS is three, all possible hydroxyl groups are substituted per AGU, and if DS is less than one, the average number of substituents per AGU is less than one. Another term (molar substitution) is preferred when the substitution is performed by chemical compounds that generate new free hydroxyl groups for further substitution. Molar substitution is defined as the average number of moles of the functional group per AGU. Commonly, molar substitution equals DS,[64] but if more than one substituent per AGU can be attached at the same position on the AGU, MS can exceed three in contrast to DS.[3,47,78-80] The determination of DS is often performed chemically[81-83] or by NMR,[62,70] dependent on the type of substituent.

1.2.2 Starch derivatives

Chemical modifications can be accomplished by either controlled acid conversion, oxidation, substitution, grafting, or cross-linking.[5,47-49,77-79]

Acid hydrolysis[5,79,84-87] is performed by suspending starch in dilute acid solution at a temperature between ambient and just below pasting temperature. The reduced molecular weight results in reduced viscosity, less swelling and a more soluble product. *Dextrinization*[5,14,79,87,88] also known as *pyroconversion* is partial depolymerization in the presence of small quantities of acid. Alternatively, there is partial hydrolysis of the molecules and recombination of starch fragments, which is known as transglucosidation.

These fragments or (pyro)dextrins exhibit low viscosity, reduced sugar content, as well as varying solubility, depending on the conversion.

Oxidation[5,79,83,86,87,89] of starch is achieved by a variety of oxidizing reagents such as hydrogen peroxide, alkaline hypochlorite, peracetic acid, persulfate, or permanganate. During the oxidation process, the starch is depolymerized, while AGUs become functionalized with carboxyl and carbonyl groups depending on the conditions used.[83,89] *Bleaching*, which introduces ≤ 0.1% carboxyl groups per AGU, is considered to be very light oxidation. Oxidized starch has a low viscosity and low temperature stability.

Cross-linked[5,26,49,78,79,90,91] starch is achieved by the reaction of two or more hydroxyl group in starch with each other, through the action of bifunctional compound. A small number of the polymer chains are chemically linked to form covalent inter- and intramolecular bridges. The introduced bridges reinforce the granule to withstand chemical and physical treatment. Cross-linked starch exhibits high viscosity. Gelatinization and swelling of the granule is inhibited, depending on the type of cross-

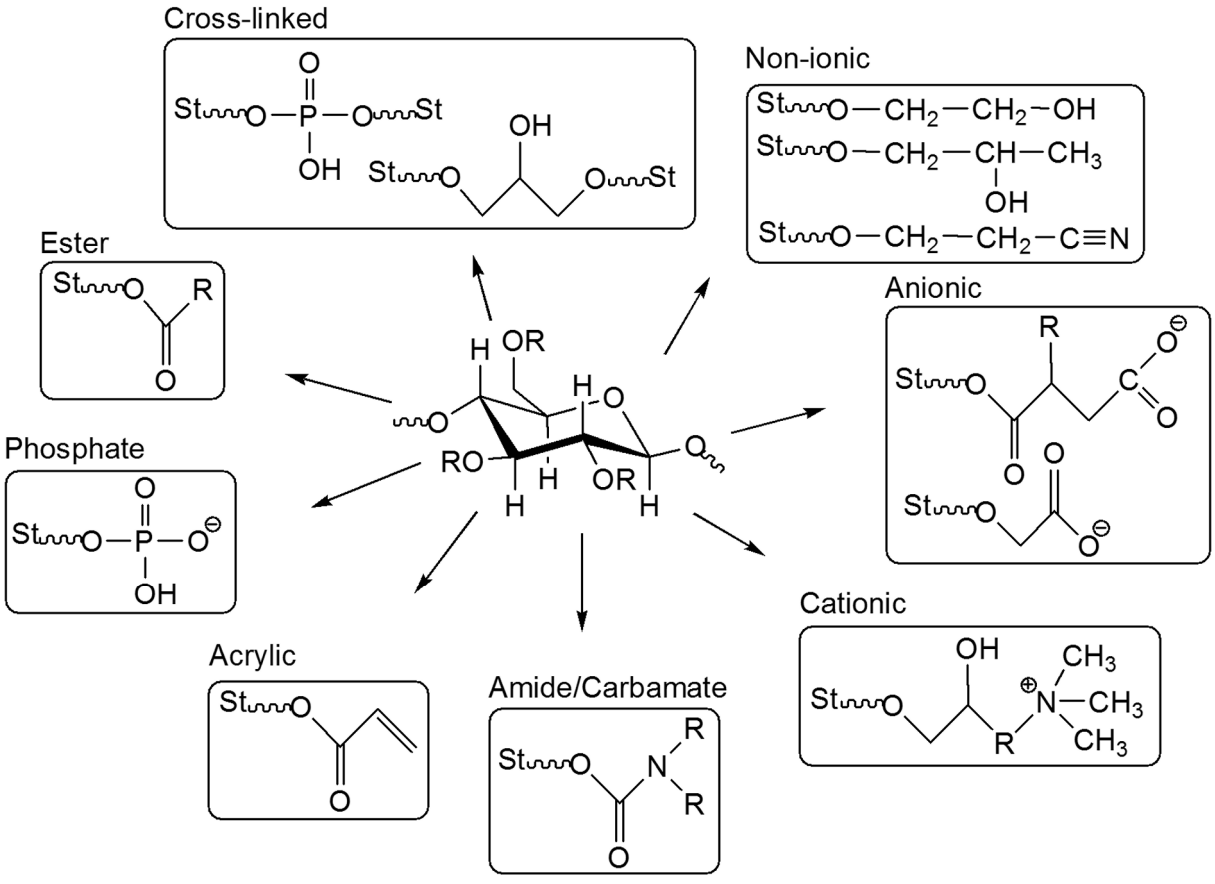


Figure 1.4. Starch modification for the production of commercial starch derivatives; St: starch; R: alkyl group.

linker. Commercial cross-linking is often performed by the reaction of bi- or polyfunctional reagents e.g. phosphorous oxychloride, sodium trimetaphosphate, epichlorohydrine, and mixtures of adipic anhydride and acetic acid (Figure 1.4).[74-76,81] Cross-linking is often performed in combination with esterification or etherification, to provide appropriate gelatinization, viscosity, and texture properties. Multiple treated starches are often used as thermoplastics[92,93], hydrogels[94] and ingredients for food products such as bread.[49,87,95]

Grafting[49,96-98] of starches is obtained by generating free radicals on the biopolymer for reaction with vinylic or acrylic monomers. Free radicals are generally formed by chemical initiation (often using ceric salts) or irradiation. Grafted starches can be used as hydrogels,[81] bioadhesive drug carriers,[99] thermoplastics and paper additives.[100-102]

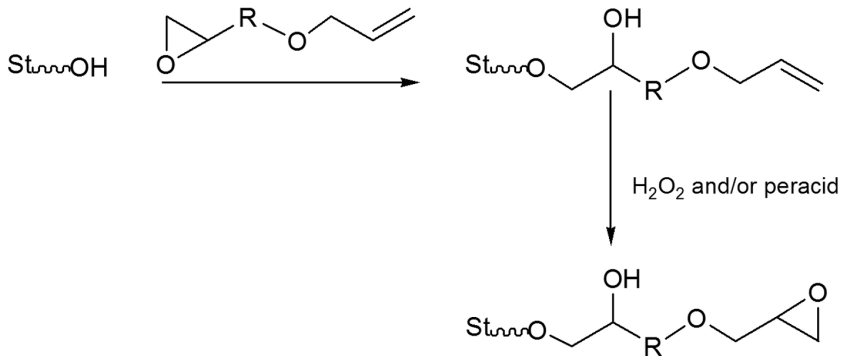
Substitution[5,49,78,79,86] of starch is commonly esterification and etherification. The reaction of starch with an etherifying or esterifying group in an alkaline medium introduces side chains in the starch structure, i.e. adding irregularities to amylopectin and especially to amylose, leading to lower gelatinization temperature. Furthermore, substitution is often performed to increase the pasting consistency and to limit retrogradation. Therefore, these starch derivatives are described as 'stabilized starches'. A broad variety of starch esters and ethers have been produced with non-ionic, anionic or cationic groups (Figure 1.4). Ionic starches are applied to improve interactions with oppositely charged surfaces e.g. succinylated or cationic starches, which can be applied as coating binders in paper industry (Table 1.1). Esterification is commonly performed with anhydrides such as acetic, vinyl or phosphoric anhydride.[50-54,57] Esterified starches are used in food applications as emulsion stabilizers, in frozen food and for encapsulation. The ether groups are mostly introduced upon reaction with alkyl halides,[69,70,72,103] cyanoethyl[49,65] or alkylene oxides.[61-64,104-108] Mostly, etherified starches are used in food applications as thickeners, in paper as additives and in textile as sizing agents.

Amongst the etherified starches are the allyl starch derivatives. The attachment of a double bond can be achieved using different allyl halides or epoxyalkyl alkenyl ethers. Generally, allyl starch derivatives are copolymerized into grafted polymers (see above). Alternatively, the allyl starch derivatives can be converted into epoxy groups.[109] The epoxy groups give the possibility to react with a wide range of ligands containing amino, hydroxyl and thiol groups. Epoxy groups can even serve as a cross-linker in starch. Epoxy starch derivatives may be used as delivery systems, stabilizators and coatings.

1.2.3 Synthesis of multifunctional granular epoxy starch derivatives

There is a strong interest in the development of granular starch based derivatives which possess chemical functional groups and allow binding with a range of nucleophilic compounds such as sulfate, polyamines, amino acids and peptides. The introduction of an epoxy group into starch gives the possibility to produce such multifunctional starch derivatives. One of the suggested approaches is to substitute the starch with an allyl group followed by epoxidation of the double bond.[109] Most synthesis of allyl starch derivatives have been performed with allyl halides in the presence of a catalyst (such as NH_4Br) at high temperature, i.e. above the gelatinization of the native starch.[81,102,110] Due to the insolubility of allyl halides in water, these syntheses with gelatinized starch have been achieved in hazardous solvents such as dimethyl sulfoxide and dichloromethane. Other carbohydrates such as cellulose have been substituted with allyl groups using similar reaction conditions.[82,108,111,112]

Allyl starch derivatives can also be obtained by the reaction of starch with epoxyalkyl alkenyl ethers such as allyl glycidyl ether (Scheme 1.1).[109] Preferable, the synthesis takes place in an alkaline solution at temperatures below 60°C. Under these conditions, granular epoxyalkyl alkenyl starch derivatives can easily be isolated and purified. Subsequently, the double bond of the epoxyalkyl alkenyl starch could be epoxidized (Scheme 1.1) to activate the starch granule for binding with other reagents. Epoxidation of double bonds is generally performed with peracids or hydrogen peroxide, preferably in water.[82,108,109,113] In a previously published method, allyl cellulose has been efficiently epoxidized using peracids in the presence of strong mineral acids as catalyst.[82] Nevertheless, the method resulted in low molecular weight cellulose derivatives, due to hydrolysis in the acidic medium.



Scheme 1.1. The synthesis of epoxy starch derivatives via a two steps chemical process; St: starch; R: alkyl group.

1.2.4 Analysis of allyl and epoxy starch derivatives

The chemical properties of allyl and epoxy starch derivatives can be investigated to gain knowledge about the starch modification. The degree of substitution (DS) of allyl groups in starch can determined using double bond titrations with mercuric acetate[114], bromine solution[82,115] and pyridinium sulfate dibromide,[102]; or using elemental analyses[81,111] or proton NMR.[62,111] For the quantification of epoxy groups in low substituted starch derivatives a very sensitive method is required. Infrared (IR) analysis and NMR are therefore not appropriate due to small intensities of the signals of epoxy groups. Mostly, the methods to determine the amount of epoxy groups are titration such as acid-base titration[116-121] or colorimetric assays.[118,122-125]

In addition to the DS, the location of the substituent group on the AGU is of interest, which can be conveniently determined by NMR[80] or gas chromatograph-mass spectrometry (GC-MS).[47,126] Besides the location on the AGU, the position and the distribution of the substituent residues within starch molecules, amylose and amylopectin, can be investigated using enzymatic degradation of the starch derivatives.[127-129] The substituents introduced into starch act as inhibitors to starch-degrading enzymes such as α-amylase, amyloglucosidase, pullulanase and β-amylase.[80,130] Analytical techniques such as high performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) can be used to analyze the enzyme-digested products which will give information about the distribution of the residues along the polymers.

1.2.5 Objective and outline of this thesis

Functionalization of starch is widely used to modify the physical and chemical characteristics of the polysaccharide to obtain desirable properties for industrial applications. There is a strong interest in the development of new granular multifunctional starch derivatives for applications as biodegradable delivery systems, stabilizators and coatings. This requires new relevant starch based derivatives which possess chemically active functional groups and allow easy binding of reagents with nucleophilic groups such as hydroxyl and amino groups. One of these new starch derivatives are epoxy starch derivatives. When epoxy groups are introduced into starch, they are able to react with compounds having a coupling, complexing or cross-linking function or with compound

having anionic or cationic groups. These starch based products can serve various purposes, e.g. flocculants, complexants, delivery systems and pharmaceuticals.

The main objective of this project was to develop and optimize a mild and environmentally friendly chemical process for the synthesis of novel highly reactive granular epoxy-starch derivatives. The goal was to understand the factors controlling the reactions and to gain knowledge about the mechanism of the starch modification. Furthermore, the properties of the modified starch were studied at the molecular level (regioselectivity, e.g. degree of substitution (DS) at the level of glucose units) and the macroscopic level (location of the substitution within the granule, the change in physicochemical properties of starch). For the control of the chemical process, experimental design was used to achieve the optimum conditions for the synthesis of new starch based derivatives.

In *Chapter 2*, the synthesis of granular maize starch with allyl glycidyl ether (AGE) is described. The granular starch derivatives, 1-allyloxy-2-hydroxypropyl-starch (AHP-starch), were characterized in terms of the degree of substitution (DS), the location of the substitution, the substituent distribution, and the granular morphology. In *Chapter 3*, an experimental design approach was taken to investigate the influence of the different process conditions on the DS of the AHP-maize starch derivatives. The chemical functionalization of maize starch with AGE alters the physicochemical properties such as gelatinization, viscosity, swelling and flow behavior, as is described in *Chapter 4*. In *Chapter 5*, the synthesis and characterization of epoxy starch derivatives is described. *Chapter 6* summarizes the data presented in this thesis and discusses the future perspectives of these new starch based derivatives.

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Chapter 2

1-Allyloxy-2-hydroxypropyl-starch: synthesis and characterization

Abstract

New reactive unsaturated starch derivatives, 1-allyloxy-2-hydroxypropyl-starches (AHP-starches), were synthesized through the reaction of waxy maize starch (WMS) or amylose-enriched maize starch (AEMS) with allyl glycidyl ether in a heterogeneous alkaline suspension containing NaOH and Na_2SO_4. The degree of substitution (DS) was determined by ^1H NMR spectroscopy and a DS of 0.20 ± 0.01 was found for both AHP-WMS and AHP-AEMS, respectively. The AHP derivatives of WMS and AEMS were further characterized by ^1H and ^{13}C NMR. It was shown that the AHP substitution was mainly located on the C-6 hydroxyl group of the glucose residues in the starch. The substitution pattern of the AHP groups along the polymer chain was randomly clustered, as determined by enzymatic digestion using pullulanase, α-amylase and amyloglucosidase, followed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy analysis of the digestion products. Using X-Ray diffraction and scanning electron microscopy, no changes in the granular morphology and crystallinity between unmodified and AHP-starches were detected.

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2.1 Introduction

Starch is one of the most abundant natural biopolymers, and large quantities thereof are chemically or physically modified to obtain desired properties for different applications in food and nonfood industries. Allyl starch derivatives form an important class of reactive starches due to the presence of highly reactive carbon-carbon double bonds.[1] These modified starches afford new possibilities for application in protective and decorative coatings for wood, glass, metal, and other surfaces, for coating and impregnating paper and textiles, and for the preparation of laminated products or rigid plastics.[2] Graft copolymers obtained from allyl starch derivatives are also used in heat-cured coatings as well as in hydrogels [3-6] and are of interest for the development of drug delivery systems.[7] Conventional syntheses of allyl starch derivatives utilize allyl halogenides in the presence of catalysts at temperatures generally above the gelatinization temperature of native starch.[2-6,8-11] As allyl chloride and allyl bromide are insoluble in water, the reactions are normally performed in polar aprotic media using hazardous solvents like dimethyl sulfoxide and dichloromethane.[1-3,8,9] Though high degree of substitution (DS up to 3), this conventional synthesis has the drawbacks of formation of large quantities of salts and the reaction products are difficult to separate due to the gelatinizing process.

In this chapter, the synthesis of 1-allyloxy-2-hydroxypropyl-starch (AHP-starch) by a synthetic route using allyl glycidyl ether (AGE) and granular waxy maize or amylose-enriched starch in an aqueous suspension under mild reaction conditions, is described. The isolated products were characterized by the determination of the degree of substitution, the location of the substituent on the anhydrous glucose units (AGUs) and the distribution of the AHP groups along the polymer chain. X-Ray diffraction (XRD) and scanning electron microscopy (SEM) were used to visualize the changes in the crystallinity and in the granular morphology of native starch and its derivatives.

2.2 Experimental section

2.2.1 Materials

Amylose-enriched maize starch (AEMS, 70% amylose; cat. S4180), racemic allyl glycidyl ether (AGE) (≥ 99%; cat. A32608), and methyl α-D-glucopyranoside (MG; cat. M9376) were purchased from Sigma-Aldrich Chemie B.V (The Netherlands). Waxy maize starch

(WMS, 0% amylose; cat. 101120) was obtained from Fluka Biochemika (Switzerland). α-Amylase (EC 3.2.1.2.) (cat. 10069, from *Bacillus subtilis*, 393 U mg^{-1}) was purchased from Fluka (Switzerland). Pullulanase (EC 3.2.1.41) (cat. M2, from *Bacillus licheniformis*, 400 U ml^{-1}) was obtained from Megazyme (Ireland). Amyloglucosidase (EC 3.2.1.3) (cat. A9268, from *Aspergillus oryzae*, 1400 U ml^{-1}) was obtained from Sigma (United States).

2.2.2 Synthesis of 1-allyloxy-2-hydroxypropyl-methyl α-D-glucopyranoside (AHP-MG)

MG (1.93 g, 9.94 mmol) and NaOH (0.0030 g, 0.08 mmol) were suspended in distilled water (6 mL) in a 20-mL carousel reaction tube and the mixture was heated to 44°C. The reaction was initiated by dropwise addition of AGE (4.75 mL, 40.0 mmol) over a period of 15 min. The mixture was stirred vigorously for 16 h. The solution was then cooled in an ice-water bath and neutralized to pH 7.0 with 2.5 M HCl. AGE was removed by extraction with ether (3 × 10 ml). Subsequent freeze-drying of the water layer and desalting with dry MeOH (2 × 10 mL) gave 1.90 g of a white powder, as a mixture of AHP-MG (15.8%) and MG (yield: 97%) (see Figure 2.2, for numbering).

^{1}H NMR (300 MHz, DMSO, δ, ppm): 5.89 (1H, H-11); 5.19 (2H, H-12); 4.71 (1H, H-1); 3.98 (2H, H-10); 3.80 (1H, H-8); 3.78 and 3.66 (2H, H-6_{MG}); 3.57 (1H, H-3); 3.56 (2H, H-$6_{AHP\text{-}MG}$) 3.54 (1H, H-5); 3.50 (2H, H-7); 3.47 (1H, H-2); 3.43 (2H, H-9); 3.33 (3H, H-13); 3.31 (1H, H-4); ^{13}C NMR (100 MHz, D_2O, δ, ppm): 133.8 (C-11); 118.3 (C-12); 99.3 (C-1); 73.1 (C-3); 72.0 (C-10); 71.6 (C-5); 71.2 (C-2); 70.8 (C-9); 70.4 (C-8); 69.9 (C-4); 62.7 (C-7); 62.5 (C-$6_{AHP\text{-}MG}$); 60.6 (C-6_{MG}); 55.0 (C-13).

2.2.3 Synthesis of 1-allyloxy-2-hydroxypropyl-starch (AHP-starch)

A general procedure for synthesis of AHP-starch was as follows: Amylose-enriched starch (25.01 g, 128 mmol AGUs, 17% w/w H_2O) was suspended in distilled water (100 mL) in a 250-mL round bottom flask. NaOH (0.48 g, 12 mmol) and Na_2SO_4 (8.8 g, 62 mmol, 42% w/w) were added and the mixture was heated to 44°C. The reaction was initiated by dropwise addition of AGE (8.77 mL, 74 mmol) over a period of 30 min. The mixture was stirred vigorously for 16 h at 44°C. The suspension was then cooled in an ice water bath and neutralized to pH 7.0 with 2.5 M HCl. After filtration (sintered glass filter, G3), the

product was washed with water (3 × 150 mL), ethanol (3 × 150 mL) and acetone (3 × 150 mL). After drying overnight in an oven at 60°C, a white powder of AHP-AEMS (28.14 g, 17% w/w water, yield: 98%) was obtained (see Scheme 2.1, for numbering).

^{1}H NMR (300 MHz, DMSO, δ, ppm): 5.90 (1H, H-11); 5.20 (2H, H-12); 4.93 (1H, H-1); 3.95 (1H, H-8 and 2H, H-10); 3.76 and 3.66 (2H, H-6); 3.50-2.95 (H-2 to H-5, H-7 and H-9); ^{13}C NMR (100 MHz, D_2O, δ, ppm): 136.2 (C-11); 117.3 (C-12); 100.9 (C-1); 79.6 (C-4); 74.1 (C-3); 72.9 (C-2); 72.5 (C-5); 71.2 (C-8 and C-9); 69.9 (C-10); 61.1 (C-6 and C-7).

The synthesis of AHP-WMS was performed with waxy maize starch (10.02 g, 53 mmol AGUs, 15% w/w H_2O) under slightly different reaction conditions than described above (Table 2.1). The reaction gave a white powder of AHP-WMS (9.10 g, 14% w/w H_2O, yield: 75%).

2.2.4 Enzymatic degradation

For the enzyme preparation, α-amylase was dissolved in deionized water purified by Millipore Milli-Q Gradient A10 (Millipore, United Kingdom), pullulanase was diluted in a sodium acetate buffer (0.01 M, pH 5.0) and amyloglucosidase was diluted in a sodium acetate buffer (0.01 M, pH 4.5), resulting in solutions containing 0.38, 0.22, and 0.14 U μL^{-1}, respectively. These enzyme solutions were used for the degradation of native starch and its derivatives. The enzymatic digestion procedure was as follows: Starch (5 mg, 0.03 mmol) was dissolved in a 1 mL sodium acetate buffer (0.01 M pH 5.0) and incubated sequentially with pullulanase (5 µL) at 40°C for 8 h, and followed by a boiling step of 10 min. Afterwards, α-amylase (5 µL) was added to the solution and kept for 8 h at 25°C. After inactivation by boiling for 10 min, the remaining solutions was incubated by amyloglucosidase (5 µL) at 55°C for 8 h and stopped by boiling for 10 min.

2.2.5 NMR spectroscopy

^{1}H and ^{13}C NMR spectra were acquired using a Bruker DPX300 300-MHz spectrometer. 2D NMR spectra were recorded on a Bruker DPX400 400-MHz spectrometer. Unless otherwise stated, all NMR measurements were carried out using DMSO-d_6 as solvent. Proton and carbon chemical shifts (δ) are given in parts per million towards tetramethylsilane (TMS).

2.2.6 Moisture contents

The moisture content determinations were carried out using the Karl Fischer method; As equipment a Metrohm 720 KFS Titrino (Metrohm, Switzerland) connected 703 Ti Stand Metrohm electrodes holder and a built-in magnetic stirrer for sample location was used.

2.2.7 High-performance size-exclusion chromatography

High-performance size-exclusion chromatography (HPSEC) was carried out in a HPLC (ThermoFinnigan, United States), with a three TSK-gel columns in series (7.5-mm × 30 cm, G4000PWXL, G3000PWXL and G2500PWXL; TosoHaas, Japan), in combination with a PWXL-guard column (40 × 6 mm;TosoH, Japan). Elution took place at 30°C with 0.2 M $NaNO_3$ at a flow rate of 0.8 mL min^{-1} and was monitored with a refractive index detector (Spectra System RI 150, Thermo Electron Corporation, Italy). The data were obtained using ChromQuest software (Dionex, United States).[12]

2.2.8 High performance anion exchange chromatography

High performance anion exchange chromatography (HPAEC) was performed on a HPLC system (Dionex, United States). The system was equipped with a quaternary gradient pump, an AS3000 autosampler complete with a helium degassing unit and an ED40 EC detector in pulsed amperometric detection (PAD) mode. The CarboPac PA1 column (2 × 250 mm; Dionex, United States) with a CarboPac PA1 guard column (2 × 50 mm; Dionex) was operated at a flow rate of 0.3 mL min^{-1} at 20°C. The gradient was obtained by solutions of mixing NaOAc (1 M in 0.1 M NaOH) with 0.1 M NaOH. After 15 min equilibration with 0.1 M NaOH, 20-µL of the sample was injected. A linear gradient to 0.5 M NaOAc in 0.1 M NaOH within 30 min was applied, followed by a linear gradient in 5 min to 1 M NaOAc in 0.1 M NaOH. Finally, the column was washed for 5 min with 1 M NaOAc in 0.1 M NaOH. The data were processed using a Chromeleon software (Dionex, United States).[12]

2.2.9 MALDI-TOF Mass Spectrometry

Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) was carried out using an ultraflex workstation (Bruker Daltonics GmbH, Germany) equipped with a nitrogen laser at 337 nm. The mass spectrometer

operated in the positive mode and was calibrated with a mixture of maltodextrins (mass range = 400-3500 Da). After a delayed extraction of 100 ns, the ions were accelerated with a kinetic energy of 20 kV. Hereafter, the ions were detected in the reflector mode. The lowest laser power required to obtain good spectra was used. The mixture of 1-μL sample and 1 μL of the matrix were dried on a sample plate. The matrix solution was prepared by dissolving 9 mg of 2,5-dihydroxybenzoic acid in a 1-mL mixture of acetonitrile:water (300 μL:700 μL).[13]

2.2.10 Scanning electron microscopy

Scanning electron microscopy (SEM) micrographs were obtained on a JEOL JSM-6300F scanning electron microscope (CT 1500 HF, Oxford instruments). The vacuum-dried samples were attached to circular silver stubs with double-sided tape on a carbon sticky tape and coated with platinum sputter using JEOL JFC-1200 fine coater (Tokyo, Japan). 'Air-dry' samples were viewed by scanning the total specimen and a representative area was photographed at magnification in ranges up to 6000 ×.

2.2.11 X-ray diffraction

X-Ray diffraction (XRD) spectra were obtained on a Philips X-pert Pro MRD apparatus (The Netherlands) under N_2. A dry sample was spread on a thin glass (~ 15 μm), which was then placed on a temperature-regulated flat copper sample stage. The relative crystallinity was determined after normalization of all recorded spectra.[14] Diffractograms were interpreted in terms of polymorphs A, B and C.[14-18]

2.2.12 The calculation of the degree of substitution

The degree of substitution (DS) of AHP derivatives was determined by the integration of ^{1}H NMR spectra using maleic acid (MA) as an internal standard. MA gives a singlet for 2 H-atoms at 6.0 ppm in ^{1}H NMR spectra. The DS, expressed as moles allyl residues per mole glucose units, was determined using the peak at 5.9 ppm attributed to H-11 from the allyl starch ether according to equation (2.1):

$$\mathrm{DS} = \frac{2 \cdot I_{AHP} \cdot x_{MA}}{x_{AGU} \cdot I_{MA}} \qquad (2.1)$$

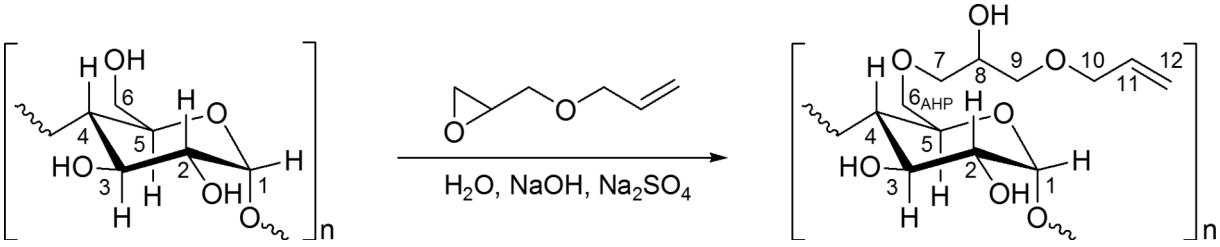
where x_{MA} and I_{MA} are the molarity and the integrated peak area of MA, respectively; I_{AHP} is the integrated peak area of AGE, and x_{AGU} is the molarity of AGUs (in starch).

Another DS determination is based on an adapted double-bond titration method.[19] This method is performed with a standard acid-base titration connected with computer program TPC2000. Briefly, allyl starch ether undergoes a reaction with $(CH_3COO)_2Hg$ in methanol. The liberated acetic acid is proportional to the amount of double bonds in AHP-starch.

2.3 Results and discussion

2.3.1 Synthesis of AHP-starch

1-allyloxy-2-hydroxypropyl (AHP) derivatives of waxy maize starch (WMS) and amylose-enriched maize starch (AEMS) were synthesized through the reaction of allyl glycidyl ether (AGE) with granular starch in an alkaline suspension in the presence of Na_2SO_4 as starch granule stabilizer (Scheme 2.1).[20-22] The reactions were performed under similar conditions as described elsewhere (Table 2.1).[23] To prevent swelling of starch under the alkaline reaction conditions, a 0.20 or 0.48 equivalent of sodium sulfate was added, respectively for WMS and AEMS respectively. Sodium hydroxide (9% mol/mol) was added to enhance the reactivity of the starch hydroxyl groups in the S_N2 reaction through formation of alkoxide anions.[5,20,24-26] The reaction temperature was kept below the gelatinization temperature of the substrate starches to facilitate the production of a starch derivative in dry, granular form.[20,21,26] After penetration of the allyl glycidyl ether into the starch granule, the reaction took place at both amylose and amylopectin fractions. The etherification of AEMS and WMS, respectively with a 0.6 equivalent and 0.3 equivalent of AGE, proceeded smoothly and resulted in products with DSs of 0.20 and 0.19 per anhydrous glucose unit (AGU), correspondingly (Table 2.1). Under these reaction



Scheme 2.1 Synthesis of 1-allyloxy-2-hydroxypropyl-(AHP)-starch.

Table 2.1 DS values of AHP-AEMS and AHP-WMS

Compound	NaOH (M)	Na_2SO_4 [a]	Ratio AGE/AGU	DS_{NMR}	$DS_{titration}$
AEMS	0.12	42	0.6	0.20 ± 0.01	0.24 ± 0.06
WMS	0.12	20	0.3	0.19 ± 0.01	0.26 ± 0.06

[a] weight percentage related to the amount of dry starch, % w/w.

conditions, this is a relatively high DS[20,26] for granular starch derivatives. It suggests a good availability of the AGE reagent inside the granule for AEMS and WMS under the heterogeneous conditions.

2.3.2 NMR characterization of AHP-starches

Figure 2.1A shows the ^{1}H NMR spectrum of AHP-AEMS. The peaks were assigned to the assumed structure of AHP-AEMS starting with the peak at 3.95 ppm which belongs to the protons H-8 (1H) and H-10 (2H). The peaks at 5.90 and 5.20 ppm were assigned to the ethylenic protons H-11 (1H) and H-12 (2H), and the peak at 4.93 ppm was attributed to the alpha anomeric proton of glucose residues (H-1, 1H). The characteristic peaks for the H-6 (2H) protons in AHP substituted and unsubstituted glucose were identified at 3.76 and 3.66 ppm. The broader peak between 3.50 and 2.95 ppm was assigned to the magnetically similar protons H-2 to H-5, H-7 and H-9. In the ^{13}C NMR (spectra not shown), the peaks characteristic for the allylic and ethylenic carbon atoms were identified at 69.9 ppm (C-10), 136.2 ppm (C-11), and 117.3 ppm (C-12), respectively. The peaks characteristic for the α-D-glucopyranosyl moiety (C-1 to C-6) and the 2-hydroxypropyl ether chain (C-7 to C-9) were identified in the ^{13}C NMR spectrum.

The peak at 5.90 ppm in the ^{1}H NMR spectrum of the AHP-starches assigned to the H-11 of the ethylenic bond in the side chain was used to quantify the DS. Maleic acid (MA) was used as internal standard (Figure 2.1B). With this method, DS values of 0.20 and 0.19 were determined for AHP-AEMS and AHP-WMS, respectively. These values for DS, based on ^{1}H NMR, were compared to the DS determined from adapted double-bond titration measurements using reported methods (Table 2.1).[19] The NMR method appeared to be more accurate than the titration method. Furthermore, the method was faster in performance. Therefore, the ^{1}H NMR approach is a more preferable method for DS determination compared to the titration method.

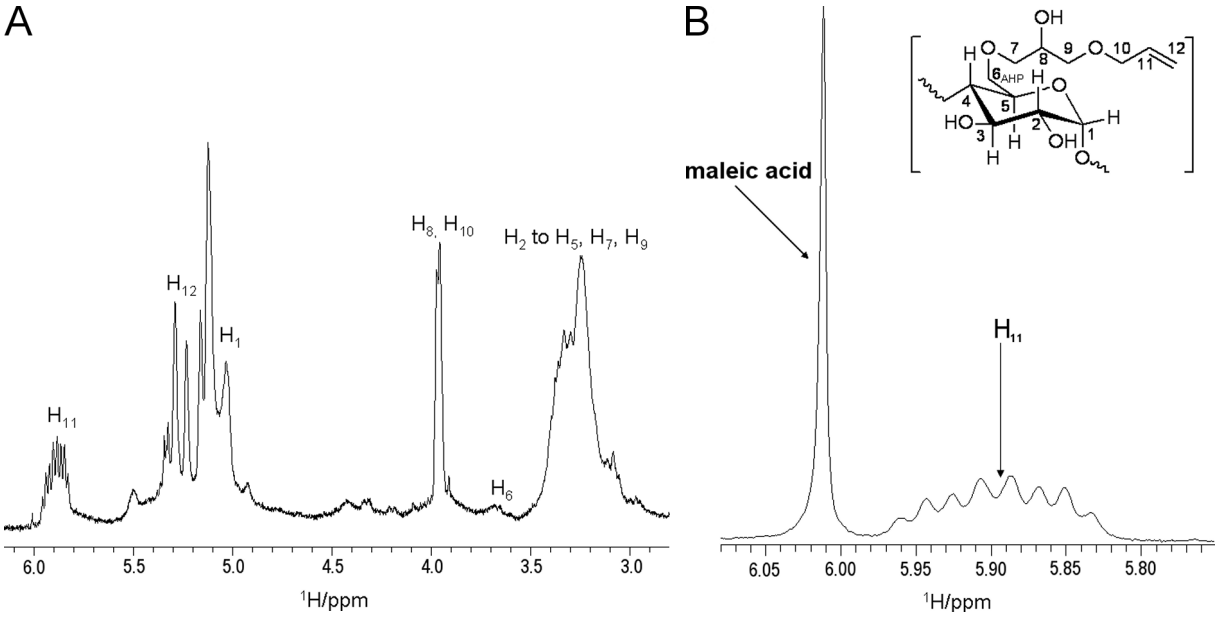


Figure 2.1 (A) ^{1}H NMR spectrum of AHP-AEMS with water suppression at 3.3 ppm. (B) ^{1}H NMR spectrum containing the peaks of maleic acid and H-11 of AHP-starch for the determination of the degree of substitution.

2.3.3 Regioselectivity of the AHP substitution

One important question addressed in this study is the location of the AHP substitution on glucose residues. According to literature, a relatively high reactivity of O-2 and O-3 compared to O-6 substitution for numerous starch and cellulose has been reported.[20] In highly acetylated starches, it was shown that the glucose residues are equally substituted in O-2 and O-3 position, whereas for hydroxypropyl starches a predominant O-2 monosubstitution is proposed.[20,27-29] In the case of AHP-starches with low degree of

Table 2.2 Chemical shift (ppm) for the ^{1}H and ^{13}C signals of the mixture AHP-MG and MG.

Number [a]	Group	^{13}C	^{1}H	Number [a]	Group	^{13}C	^{1}H
11	CH	133.8	5.89	9	CH_2	70.8	3.50
12	CH_2	118.3	5.19				3.43
1	CH	99.3	4.71	4	CH	69.6	3.31
3	CH	73.1	3.57	7	CH_2	62.7	3.53
10	CH_2	72.0	3.98				3.48
5	CH	71.6	3.55	6_{AHP-MG}	CH_2	62.5	3.56
2	CH	71.2	3.47	6_{MG}	CH_2	60.5	3.78
8	CH	70.4	3.80	13	CH3	55.0	3.33

[a] Numbering of atoms as shown in figure 2.2; 6_{MG} and 6_{AHP-MG} indicate the C-6 atom in MG and AHP-MG, respectively.

etherification, using the method described in this study, the ^{1}H NMR, ^{13}C NMR and 2D NMR analysis suggest that the glucose moiety is preferentially substituted at the O-6 position. To validate the NMR information for O-6 substitution, AHP-MG was synthesized and characterized. MG was chosen as a model structure for the glucose residues in starch. The synthesis of AHP-MG has been performed with a low molar ratio MG and AGE to favor a specific substitution. The reaction product with an overall DS of 0.11 contained 15.8% AHP-MG, and was therefore a good monomer model of the AHP-starch with a DS of 0.20.

Apart from ^{1}H NMR, ^{13}C NMR, DEPT90 and DEPT135 spectra, the structure of AHP-MG was studied using ^{1}H-^{13}C HETeronuclear chemical shift CORrelation (HETCOR, one band correlation), ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC), and ^{1}H-^{1}H COrrelation NMR SpectroscopY (COSY). Via 1D NMR, the chemical shift for both carbon atoms and the hydrogen bonds were obtained (Table 2.2). The matching hydrogen atoms were elucidated with ^{1}H NMR and HETCOR. The sequence of the carbon atoms was obtained via COSY and HMBC spectra. Using HMBC, a specific correlation showed an O-6 substitution. This substitution was elucidated by the

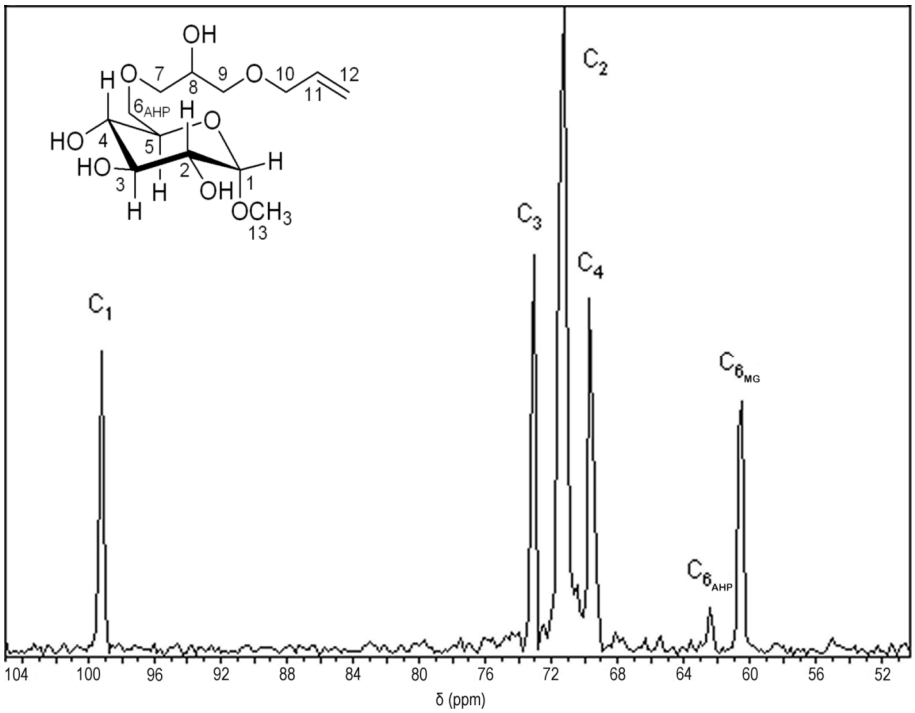


Figure 2.2 A one-dimensional cross section of the proton frequency dimension of C-5 (3.55 ppm) of the HMBC spectrum of methyl α-D-glucopyranoside (MG) and AHP-MG-mixture.

HMBC section at the C-7 proton frequencies, 3.48 and 3.53 ppm, and C-5 proton frequency 3.55 ppm. In the cross section of proton frequency of C-5, the correlations between H-5 and the matching C-atoms are visible (Figure. 2.2). The important peak is at 62.5 ppm, which appeared to be a CH_2 group according to the DEPT135. After correlation of all the spectra, we can conclude that the peak at 62.5 ppm must be assigned to the C-6_{AHP-MG}, the primary carbon in AHP-MG. Similarly, the cross section of proton frequencies of C-7 elucidated the correlations between H-7 and C-8, C-6_{MG} as well as the H-7 and C-6_{AHP-MG} correlation.

This analysis shows that the AHP substitution of MG is regioselective, and was preferentially located at the O-6 position of glucose. Substitution at C-2 and C-3 position cannot be ruled out since their respective correlations might be concealed in the broad signals of the HMBC analysis. Less steric hindrance at the O-6 position may well explain the regioselectivity in this modification reaction and type of substitution instead of, in general, O-2 or O-3 position.[20] Because of the agreement between the results of the NMR analysis of AHP-starch derivatives and AHP-MG, we conclude a predominantly O-6 substitution of glucose residues in AHP-starch. Other techniques such as HPLC analysis and gas chromatograph-mass spectroscopy might provide more complete evidence for the position of AHP group on the glucose units. These techniques require sample preparation, such as permethylation and hydrolysis of the modified starch.[30]

2.3.4 Distribution of allyl glycidyl ether groups along the polysaccharide chains

To determine the distribution of the AHP groups along the polysaccharide chains, the modified starches were subjected to enzyme degradation. (Starch)-degrading enzymes hydrolyze the glycosidic linkages between the glucose residues in the polysaccharide chain in a defined way. The introduction of the AHP groups may sterically hinder the action of the amylolytic enzymes.[25] Knowledge about the enzyme degradation products of AHP-starch can give significant information about the distribution of the AHP substitution along the polymer chains. For the enzymatic digestion of AHP-starches, three (starch)-degrading enzymes with different cleavage specificity were used: pullulanase, α-amylase and amyloglucosidase. Pullulanase is a debranching enzyme, which hydrolyzes α-(1,6)-linkages of amylopectin to produce linear chains.[31] α-Amylase is an endo-enzyme

which hydrolyzes polysaccharides randomly at α-(1,4)-D-glucosidic linkages to produce glucose and oligosaccharides containing two to seven glucose residues, and amyloglucosidase is capable of completely hydrolyzing both α-(1,4)- and α-(1,6)-linkages in polysaccharides, through an exo-mechanism from the non-reducing terminal residues, to produce β-D-glucose.[31] The action of the selected enzymes on an amylopectin chain is illustrated in Figure 2.3.

AEMS containing 70% amylose and 30% amylopectin, WMS (mainly amylopectin) and the corresponding AHP derivatives were subjected to sequential enzymic hydrolysis using pullulanase, α-amylase, and amyloglucosidase. HPSEC elution profiles of the digested samples showed a complete conversion of AEMS and WMS into glucose and small oligomeric fragments (not shown). Higher oligomers resistant to further enzymatic digestion were present in AHP-AEMS and AHP-WMS, as clearly seen from HPAEC elution profiles (Figure 2.4). This indicates that α-amylase and amyloglucosidase digestion was hindered by AHP groups. The peaks could not be assigned because the enzymatically degraded fragments of the derivatives may have different charges, leading to different retention times.

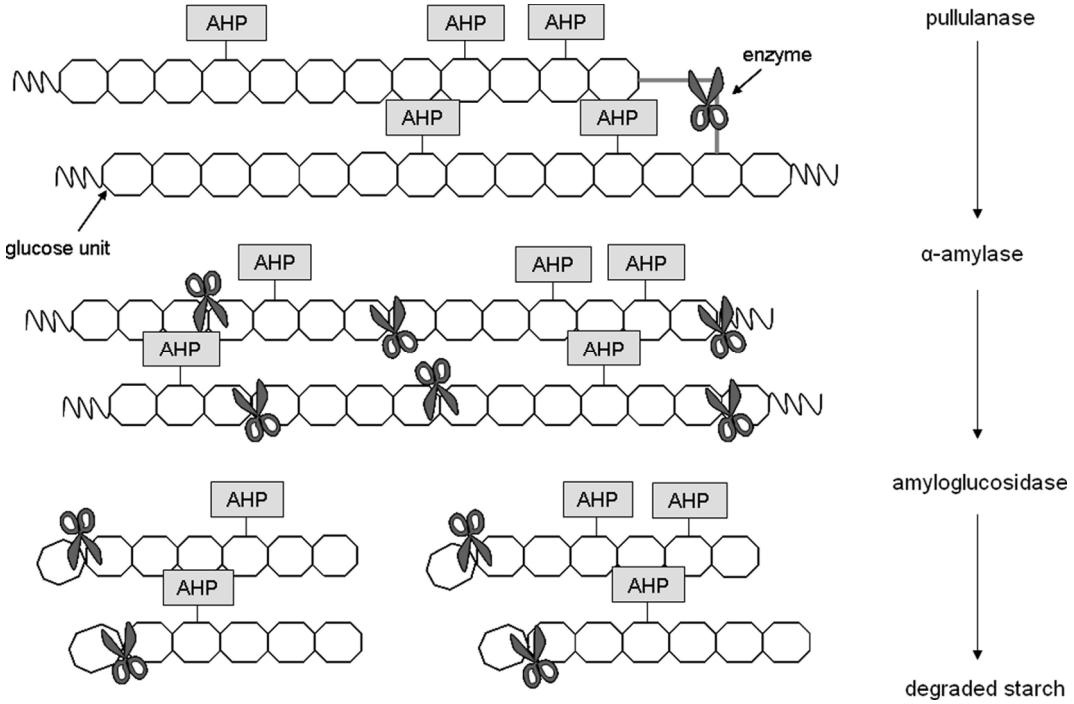


Figure 2.3 Diagram of the enzymatic digestion of starch with three enzymes: pullulanase, α-amylase and amyloglucosidase.

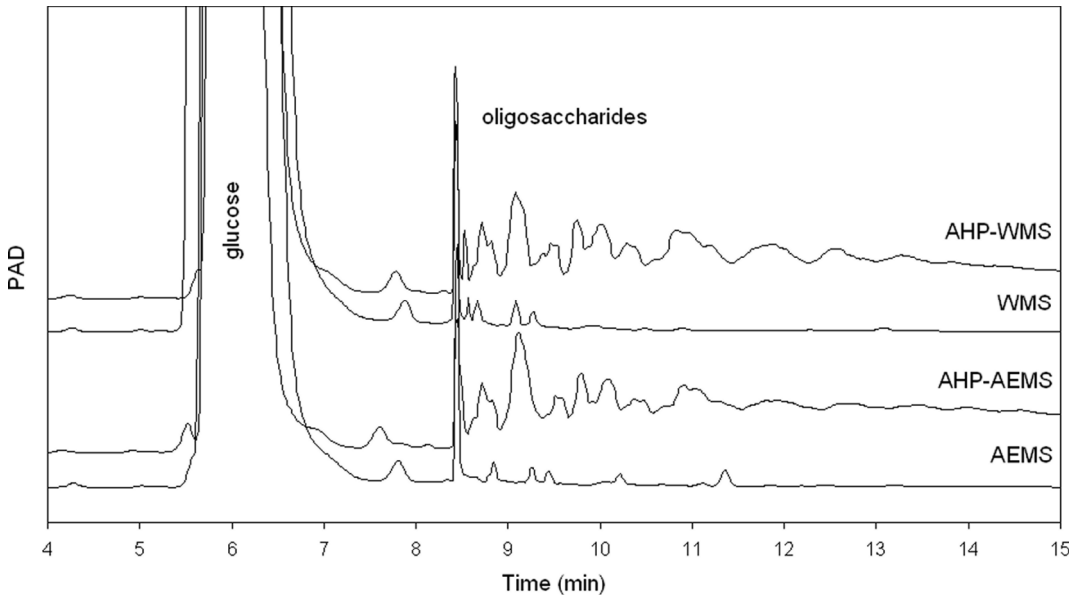


Figure 2.4. HPAEC elution profiles of the pullulanase, α-amylase and amyloglucosidase hydrolysates of AEMS and WMS and their derivatives, AHP-AEMS and AHP-WMS.

The mass distribution of the enzyme-digested hydrolysates of AHP-AEMS and AHP-WMS was determined by MALDI-TOF MS. A significant difference was observed between the substitution pattern of AEMS and WMS derivatives. The MALDI-TOF mass spectrum of "AHP-AEMS digests" shows a mixture of enzyme-resistant AHP-substituted oligosaccharides with degrees of polymerization (DP) ranging from three to ten (Figure 2.5A). The following substituted oligosaccharides were identified: maltotriose (DP3) with one and two AHP groups, DP4 and DP5 with one, two and three AHP groups, DP6 with two and three AHP groups, DP7 and DP8 containing two, three and four AHP groups, DP9 containing three and four AHP groups, and DP10 containing four AHP groups. The DS of the modified oligosaccharides derived from AHP-AEMS varied with the DP of the carbohydrate oligomer. The highest substitution for the oligomers (theoretical DS values of 0.60, 0.66 and 0.75) was observed for tri-, tetra- and pentaoligosaccharides, respectively. Larger fragments are indicative for a more clustered AHP substitution, with lower theoretical DS values ranging between 0.29 and 0.57. The MALDI-TOF mass spectrum for AHP-WMS hydrolysates shows smaller oligomers (DP ranging from three to seven) with low DS (Figure 2.5B). The following oligomers were identified: DP3, containing one AHP group (theoretical DS value of 0.33), DP4 and DP5, containing one and two AHP groups (theoretical DS between 0.20 and 0.50), and DP6 and DP7, containing two and three AHP groups (theoretical DS between 0.28 and 0.50).

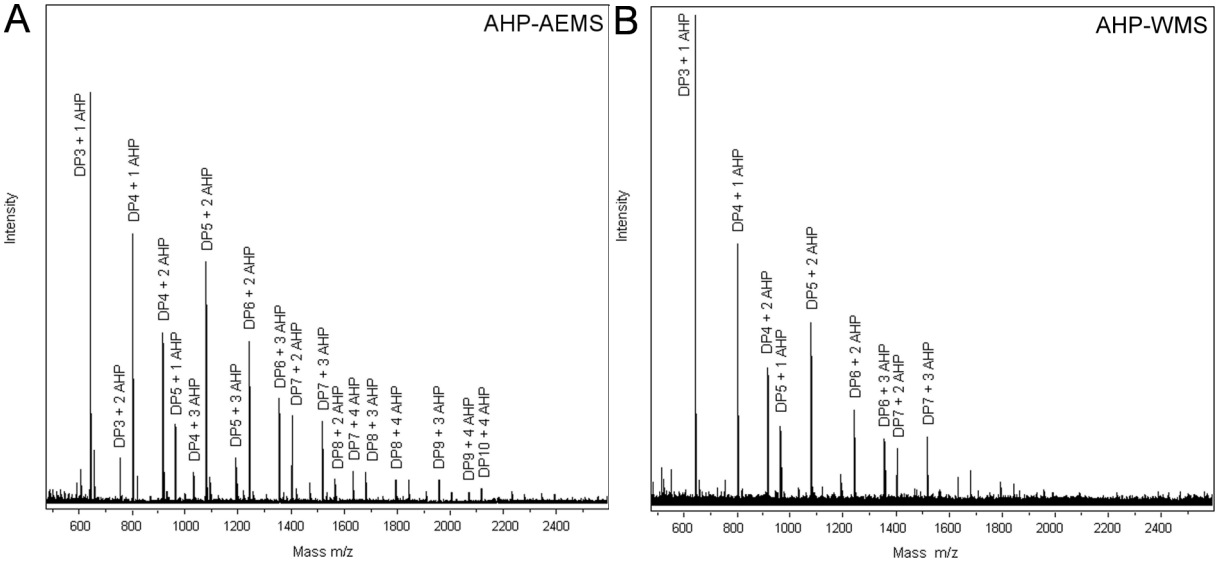


Figure 2.5 MALDI-TOF mass spectrum of the enzyme (pullulanase, α-amylase and amyloglucosidase) hydrolysates of A) AHP-AEMS and B) AHP-WMS (AHP: 1-allyloxy-2-hydroxypropyl group). (DP: degree of polymerization).

Both derivatives have an average DS of 0.20, corresponding to one AHP group per five glucose residues. The minimum indicative DS substitution required for enzyme hindrance is 0.20, although we also indicated that a hindrance for DS 0.28 and 0.43 (two and three AHP groups per seven glucose residues) appeared. Therefore, these results show that the enzyme-resistant carbohydrate residues contain at least one AHP group and confirm that substitution of the polysaccharide chain prevents enzyme-induced degradation due to sterical hindrance.

The size and the substitution pattern of the enzyme-resistant oligomers show significant differences between the susceptibility for AHP substitution of amylose and amylopectin. Amylopectin, the main component of WMS, is more uniformly substituted and the AHP-groups are homogeneously distributed throughout the polymer, essentially non-clustered. On the contrary, the AEMS starch, containing 70% amylose and 30% amylopectin shows a more heterogeneous distribution of AHP groups, as illustrated by the heterogeneity of the oligomers formed upon enzyme degradation. In the modified oligosaccharides derived from AHP-AEMS, we have identified oligomers with low DS values, similar to those generated from AHP-WMS, and highly substituted larger oligomers with clustered AHP groups. We assume that the larger fragments with high and clustered AHP substitution are degradation products of AHP amyloses. On the basis of these results, we further assume that, in general, the amylose population is more clustered

substituted than the amylopectin population and the distribution of the AHP groups throughout the amylose chain is randomly clustered.

The ^{1}H NMR results indicate that AHP-AEMS and AHP-WMS are equally substituted by AGE, although their substitution patterns are different. According to previous substitution distribution studies, substitutions takes place preferentially in the amorphous regions of the starch granule, containing amylose and the branched part of amylopectin. The crystalline regions, containing amylopectin, are only partially accessible for substitution.[20,24,25,32] Assuming that the AHP substitution of amylose taking place to a larger extent than that of amylopectin, this suggests an easier penetration of the AGE reagent throughout the amorphous regions of the starch granules, and only partial penetration in the crystalline regions.

2.3.5 Morphological properties

The effect of substitution on starch crystallinity and type of packing was investigated using X-ray diffraction (XRD). Because XRD traces of AEMS and WMS and their AHP derivatives showed similar pattern, we show only the XRD spectra of AEMS and AHP-AEMS as representative (Figure 2.6). An inspection of XRD spectra showed hardly any differences in the type of packing between native starches and their derivatives after the etherification. Furthermore, the crystalline and amorphous parts seem to be similar in structure for native starches and their corresponding AHP derivatives.

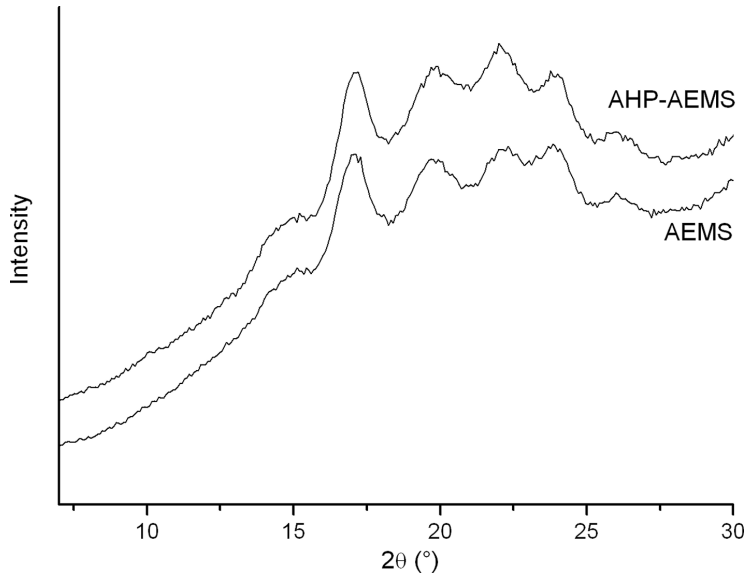


Figure 2.6. X-ray diffraction patterns of AEMS, and AHP-AEMS.

With SEM (Figure 2.7), the particle appearance of the dry starch samples was investigated. The appearance of WMS was portrayed with distinct granules, having mixed shapes, with some almost spherical, some cubical, sometimes 'honeycomb' shapes with irregular, but smooth surfaces (Figure 2.7A), as reported before.[33] Likewise, the appearance of AEMS was described as being smooth and uniform (Figure 2.7B).[34] The size and appearance of the granules from AHP-AEMS and AHP-WMS are very similar to those of their native starches (Figure 2.7C and 2.7D). However, the SEM micrographs show that the surface of the granule from AHP-AEMS and AHP-WMS are rougher and more porous than that of AEMS and WMS. It suggests that etherification of AEMS and WMS starch generates only minor surface modification upon reaction.

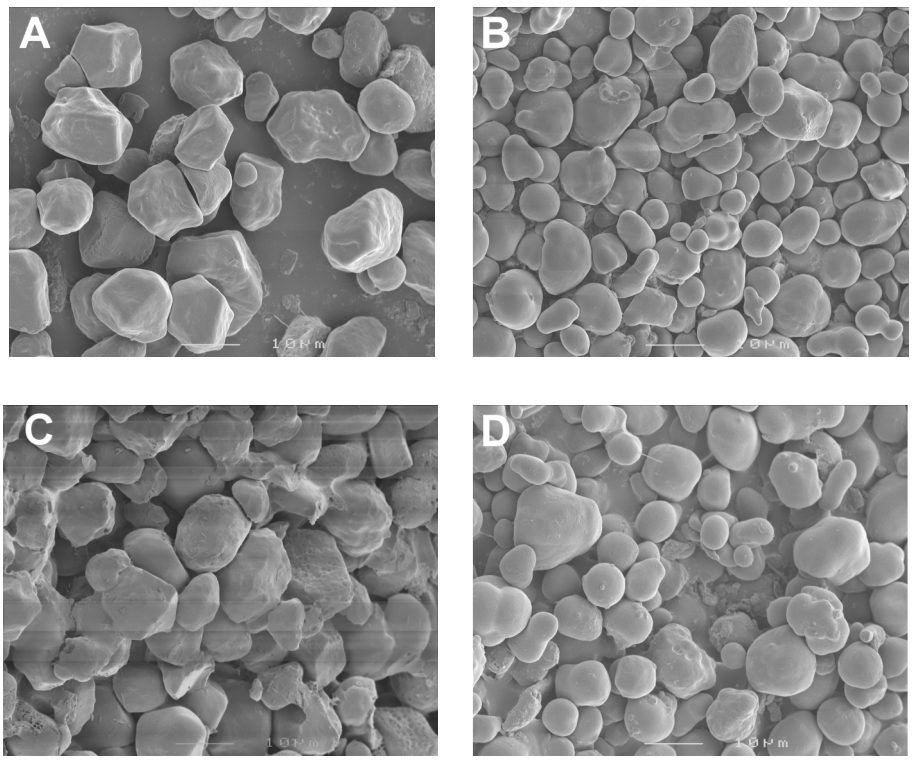


Figure 2.7. SEM images of (A) WMS, (B) AEMS, (C) AHP-WMS and (D) AHP-AEMS. The micrographs were taken at a magnification of 1500 ×. Scale bar is 10 μm.

2.4 Conclusions

Reactive unsaturated starch derivatives (AHP-starches) were synthesized by alkali-mediated etherification of starch with AGE. Granular AHP-starches with relatively high DS were produced under the alkaline reaction conditions. The modification, as described, does not significantly affect the granular morphology. The reaction is regioselective and predominantly O-6 substituted derivatives are obtained. AHP groups are clustered and randomly distributed along the polysaccharide chain. The substitution pattern depends on the type of starch, with amylose-containing starches yielding derivatives having more clustered AHP groups. The method is particularly suitable for functionalizing nongelatinized starches.

Acknowledgments

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Chapter 3

Optimization of the synthesis of 1-allyloxy-2-hydroxypropyl-starch through statistical experimental design

Abstract

The synthesis of 1-allyloxy-2-hydroxypropyl starches (AHP-starches) was studied using a statistical experimental design approach. The etherification of two different granular maize starches, waxy maize and dent maize starch, with allyl glycidyl ether (AGE) in a heterogeneous alkaline suspension was investigated. The optimal reaction conditions were found via experimental design and the obtained response factor, e.g. the degree of substitution (DS) of the starch hydroxyl groups, was statistically evaluated. The effects of six process factors on DS, namely the starch concentration, the reaction time, the temperature, and the amount of NaOH, Na_2SO_4, and AGE were investigated. The statistical analysis showed significant impact of the temperature, the amount of NaOH and the amount of AGE on the DS for both starches. Optimum conditions for the highest DS for waxy maize starch were: 0.0166% w/w AGE (based on dry starch (ds)) and 1.0% w/w NaOH (ds) at 34°C in 4 h; on dent maize starch, these were 0.0099% AGE (ds) and 1.0% NaOH (ds) at 37°C in 16 h.

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3.1 Introduction

Starch is the most widely produced industrial polysaccharide. Starch production keeps on growing continuously because of the steadily increasing demand in food and non-food applications. In 2005, 58 million tons of starches were produced worldwide.[1] Because of its abundance and properties, starch has been of interest in pharmaceuticals, biodegradable plastics, paper, etc. Chemical and enzymatic modification is a way to alter the structure of starch in a controllable manner, to enhance functional properties of starches and to extend the number of applications.[2,3] Etherification and esterification are the most common chemical derivatizations.[1,4] The properties of modified starches are depending upon the number, the distribution and the nature of the substituents [3,5] and those with a degree of substitution (DS) up to 0.20 are of commercial importance.[3]

In the previous chapter, we have synthesized and characterized etherified granular starch derivatives containing allyl groups.[6,7] We have shown that granular maize starch could be etherified easily by reacting allyl glycidyl ether (AGE) in heterogeneous suspension in the presence of Na_2SO_4 and NaOH as, respectively, starch granule stabilizer[4-6] and reaction enhancer[6,8] leading to DS up to 0.2. The reaction temperature was kept below the gelatinization temperature of the substrate starches to facilitate the production of starch derivatives in granular form.[4,5,9] The etherification of starch with AGE changed not only the chemical structure of granular maize starches, but also the physicochemical properties were altered.[7] Waxy maize and dent maize starch derivatives show higher final and pasting viscosity, better swelling power and solubility than their native counterparts.

The modification of starch most likely depends on a large number of reaction conditions, including the temperature and the reagent concentration. Moreover, the condition of the starch granule, i.e. the product quality, cannot be predicted with a physical model because of its heterogeneity. For the optimization of this kind of chemical processes, an experimental design approach, including statistical analysis, can be used.[10-14] This method uses a minimum of experiments to obtain maximum information about the experimental parameters. Mostly, the experimental design is used to investigate reaction conditions in order to determine the most important process factors and secondly to determine the optimum conditions for a chemical process.

In this study, the optimization of the etherification of granular starch with AGE to achieve a high degree of substitution is studied. The synthesis of the new material 1-allyloxy-2-hydroxypropyl-starch derivatives of dent maize starch and waxy maize starch is investigated with the aim to find the optimum process conditions. A two step approach was followed: firstly the process parameters were identified (screening phase), and secondly the processes were optimized based on these parameters (improving phase). In the screening phase, the starch concentration, the reaction time, the temperature, the amount of NaOH, the amount of Na_2SO_4 and AGE were evaluated as reaction variables. To the best of our knowledge, this is the first time that such a study to optimize process parameters for starch etherification with allyl glycidyl ether using response surface analysis is reported.

3.2 Experimental section

3.2.1 Materials and methods

Dent maize starch (27% w/w amylose, MS), and waxy maize starch (0.9% w/w amylose, WMS) were gifts from Tate & Lyle Food and Industrial Ingredients Europe (The Netherlands). Allyl glycidyl ether (≥ 99%, cat. A32608), sodium hydroxide standard solution (0.5065 N, cat. 319503) and dimethyl sulfoxide-d_6 (99.5%, cat. 175943) were purchased from Sigma-Aldrich Chemie B.V (The Netherlands). Sodium sulfate (99%, extra pure, anhydrous, cat. 19664) was obtained from Acros Organics (Belgium).
Parallel reactions of the experimental design were performed in a Radley's carousel of 12 reactors equipped with 20-mL tube and magnetic stirrings bars (medium cross type). ^{1}H NMR spectra were recorded with a Bruker DPX300-MHz spectrometer at 45°C. All ^{1}H NMR measurements were performed in DMSO-d_6 containing maleic acid as an internal standard. Prior to the experiment, the moisture content of WMS and MS was determined using a Karl Fisher titrator as described in previous chapter. Design-Expert Version 7.1.3 (Stat-Ease Co. Minneapolis, MN, USA) was used to conduct the statistical analyses, surface plotting and optimization.

3.2.2 Experimental procedure

The synthesis of 1-allyloxy-2-hydroxypropyl-starch (AHP-starch) proceeds via a one-step reaction as described in previous studies.[6,7] As an example, experiment nr 1 of waxy

maize starch is taken to explain the procedure. For the other experiments the same procedure was used with adjusted amounts of chemicals, time and temperature, as given in Table 2. 2.22 g of waxy maize starch (12 mmol anhydrous glucose units, 10.8% w/w H_2O, 200 g kg^{-1} starch suspension (C_{starch})) and Na_2SO_4 (0.1 g, 0.7 mmol, 5% w/w dry starch (ds)) were added to 0.02 M NaOH-solution (10 mL, 0.2 mmol, 0.4% w/w ds) in a reaction tube. The reaction vessel (20-mL tube with a magnetic stirrer) was placed in the carousel thermostated at 20°C. After heating to 20°C, allyl glycidyl ether (AGE; 0.34 mL, 2.8 mmol, 0.0016% w/w ds) was added dropwise to the starch suspension and the mixture was stirred vigorously for 4 h. The reaction was stopped by cooling on ice-water. The reaction mixture was neutralized with 2.5 M HCl (pH = 7) and washed on a glass filter (G3) with H_2O (3 x 15 mL), ethanol (3 x 15 mL) and acetone (3 x 15 mL). The product yield (80%) was determined after drying in an oven at 60°C for one night. The product yield was defined as the ratio of the weight of the dry product to the weight of the dry starch and the amount of AGE.

The moisture content of the products was determined by drying: 100 mg sample was added in a vial (1.5 mL and placed in a vacuum-oven at 60°C for three days, until constant weight. The degree of substitution (DS) of the product was determined using ^{1}H NMR and calculated using the equation described in the previous chapter. For this, a sample of AHP-starch (~ 8 mg) was dissolved in DMSO-d_6 (1 mL) and heated to 45°C in order to obtain a higher resolution in the ^{1}H NMR spectra.

3.3 Results and Discussion

The goal of the research described in this study was to determine the influence of different process conditions on the degree of substitution (DS) of 1-allyloxy-2-hydroxypropyl-starch (AHP-starch) obtained during the modification with allyl glycidyl ether (AGE). The influence of the process factors on the DS was investigated with the aid of an experimental design approach. In this approach several reaction parameters are varied between well chosen boundaries and the results are statistically analyzed. Low DS etherified starch was synthesized in an aqueous slurry reaction, i.e. the starch modification was performed with high starch concentrations (20-40% w/w.) as usually applied in industrial processes.[1,2,4,15,16] Sodium hydroxide was added to enhance the AGE reaction with the hydroxyl groups of starch.[6,9,16-18] Consequently, to prevent swelling of starch under the alkaline reaction conditions, sodium sulfate was added as starch stabilizer.[2,4,5]

Furthermore, the etherification reaction was performed at temperature below 50°C to avoid gelatinization of starch.[2,3,6,7] A two stage approach was followed for experimental design: in the first stage the significant parameters were identified (screening phase) and in the second stage the process was optimized based on the significant parameters found in the screening phase (improving phase).

3.3.1 Screening phase

Based on our experience and reported literature,[6,9,16-18] six process factors, which were expected to have a significant influence on the DS, were selected for the screening phase. The effect of the following factors was investigated: the starch concentration (C_{starch}, g kg^{-1} starch suspension), the reaction time (h), the temperature (°C), the amount of NaOH (as weight percentage related to the amount of dry starch, % ds), the amount of Na_2SO_4 (% ds) and the amount of AGE (% ds). The different reaction factors, inclusive boundary values, are presented in Table 3.1. All treatments were performed in random order and data were analyzed using a response surface regression procedure.

Table 3.2 gives an overview of the performed experiments. The experimental conditions were generated by the software package "Design Expert 7.1.3" in which was chosen for Fractional Factorial Design (FFD). FFD generated a subset of 32 experiments of a full fractional design (64 exp.). The center points, which have mid-spec factor settings, were repeated three times, to get an estimation of the reproducibility of the performed experiments. The first 35 experiments of the design scheme were performed for each of the two maize starches used, waxy maize starch (WMS) and dent maize starch (MS). The obtained response, for the specification part of the screening, was the DS. The obtained DS values are also given in Table 2 (exp. 1-35).

Table 3.1. Experimental design: Six variables at three levels.[a]

	min-max levels		
Variables	-1	0	1
C_{starch} (g kg^{-1} slurry) [b]	200	300	400
Reaction time (h)	4	10	16
Temperature(°C)	20	34	48
NaOH (% ds) [c]	0.4	0.7	1.0
Na_2SO_4 (% ds) [c]	5	20	35
AGE (% ds) [c]	0.0016	0.0091	0.0166

[a] The levels are indicated with 1, 0 and -1. [b] Starch concentration. [c] Variables are calculated as % (w/w) on dry starch (ds); Molar ratio of allyl glycidyl ether (AGE), NaOH and Na_2SO_4 per anhydrous glucose units are respectively, 0.24-2.36, 0.02-0.04 and 0.07-0.40 mol mol^{-1}.

Table 3.2. Experimental design and responses for the DS values of WMS and MS (DS_{starch}).

Entry	C_{starch} [c]	Time (h)	T (°C)	NaOH [d]	Na_2SO_4 [d]	AGE [de]	DS_{WMS}	DS_{MS}
Screening phase [a]								
1	200	4	20	0.4	5	0.0016	0.000	0.000
2	400	4	20	0.4	5	0.0166	0.035	0.000
3	200	16	20	0.4	5	0.0166	0.000	0.000
4	400	16	20	0.4	5	0.0016	0.000	0.000
5	200	4	48	0.4	5	0.0166	0.031	0.000
6	400	4	48	0.4	5	0.0016	0.019	0.000
7	200	16	48	0.4	5	0.0016	0.023	0.007
8	400	16	48	0.4	5	0.0166	0.067	0.059
9	200	4	20	1.0	5	0.0166	0.029	0.002
10	400	4	20	1.0	5	0.0016	0.000	0.002
11	200	16	20	1.0	5	0.0016	0.011	0.001
12	400	16	20	1.0	5	0.0166	0.026	0.012
13	200	4	48	1.0	5	0.0016	0.015	0.006
14	400	4	48	1.0	5	0.0166	0.075	0.034
15	200	16	48	1.0	5	0.0166	n.d.[f]	n.d.[f]
16	400	16	48	1.0	5	0.0016	0.063	0.107
17	200	4	20	0.4	35	0.0166	0.003	0.000
18	400	4	20	0.4	35	0.0016	0.000	0.000
19	200	16	20	0.4	35	0.0016	0.000	0.000
20	400	16	20	0.4	35	0.0166	0.006	0.000
21	200	4	48	0.4	35	0.0016	0.016	0.002
22	400	4	48	0.4	35	0.0166	0.007	0.000
23	200	16	48	0.4	35	0.0166	0.131	0.107
24	400	16	48	0.4	35	0.0016	0.044	0.081
25	200	4	20	1.0	35	0.0016	0.000	0.000
26	400	4	20	1.0	35	0.0166	0.040	0.007
27	200	16	20	1.0	35	0.0166	0.015	0.010
28	400	16	20	1.0	35	0.0016	0.010	0.006
29	200	4	48	1.0	35	0.0166	0.136	0.032
30	400	4	48	1.0	35	0.0016	0.189	0.028
31	200	16	48	1.0	35	0.0016	0.128	0.069
32	400	16	48	1.0	35	0.0166	0.042	0.185
33	300	10	34	0.7	20	0.0091	0.113	0.025
34	300	10	34	0.7	20	0.0091	0.085	0.053
35	300	10	34	0.7	20	0.0091	0.115	0.024
Improving phase [b]								
36	300	4	45	0.4	20	0.0016	0.005	0.011
37	300	4	45	0.4	20	0.0016	0.010	0.012
38	300	4	45	0.4	20	0.0166	0.003	0.008
39	300	4	45	0.4	20	0.0166	0.004	0.003
40	300	18	45	0.4	20	0.0016	0.008	0.015
41	300	16	45	0.4	20	0.0016	0.012	0.005

Entry	C_{starch} [c]	Time (h)	T (°C)	NaOH [d]	Na_2SO_4 [d]	AGE [de]	DS_{WMS}	DS_{MS}
42	300	16	45	0.4	20	0.0166	0.022	0.009
43	300	16	45	0.4	20	0.0166	0.012	0.023
44	300	4	45	1.0	20	0.0016	0.004	0.004
45	300	4	45	1.0	20	0.0016	0.014	0.011
46	300	4	45	1.0	20	0.0166	0.019	0.012
47	300	4	45	1.0	20	0.0166	0.011	0.024
48	300	16	45	1.0	20	0.0016	0.044	0.020
49	300	16	45	1.0	20	0.0016	0.036	0.026
50	300	16	45	1.0	20	0.0166	0.019	0.011
51	300	16	45	1.0	20	0.0166	0.021	0.017
52	300	10	45	0.7	20	0.0000	0.000	0.000
53	300	10	45	0.7	20	0.0182	0.004	0.019
54	300	2	45	0.7	20	0.0091	0.011	0.008
55	300	22	45	0.7	20	0.0091	0.010	0.050
56	300	10	45	0.1	20	0.0091	0.005	0.011
57	300	10	45	1.3	20	0.0091	0.030	0.035
58	300	10	45	0.7	20	0.0091	0.009	0.024
59	300	10	45	0.7	20	0.0091	0.009	0.017
60	300	10	45	0.7	20	0.0091	0.007	0.019

The product yield of the screening phase and improving phase was respectively between [a] 34-86% and [b] 34-90%. Variables are calculated as [c] g kg^{-1} slurry or [d] % (w/w) on dry starch. [e] Allyl glycidyl ether. [f] Not determined.

As can be seen in Table 3.2 the DS values in the screening phase are between 0.000 and 0.189 showing that high substitution of granular starch was obtained under the performed reaction conditions. The product yield was found to be between 34-86%. The DS value of condition number 15 has not been determined. The combination of the different factors resulted in gelatinized starches. This can be explained by the fact that the gelatinization temperature of starch in water is determined by several factors such as temperature, shear stress, amount of NaOH and amount of Na_2SO_4. It is common knowledge that the gelatinization temperature will decrease if the amount of NaOH and the shear stress are increasing, and the gelatinization temperature will increase with the increase of Na_2SO_4. Therefore, Na_2SO_4 helps to prevent gelatinization of starch during the reaction. Not gelatinized particles are favorable because gelatinized particles will give a lot of practical difficulties during purification, further modifications and processing. Although 5% (ds) of Na_2SO_4 was used in the experiment 15, this amount was not sufficient to compensate for the effect of the other factors on gelatinization of the starch. Since gelatinized starch is more accessible for AGE groups than granular starch, resulting in a higher DS, experiment 15 is not used in the model.

The responses of the different samples were analyzed using the statistical module of the experimental design program. The responses are statistically described with second-order interactions (2^m), which are characteristic for a FFD. This approach allows to estimate the main effects and all interactions up to m (m is six variables in this study). The generalized regression model was used, as shown in equation (3.1).

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum \sum_{i<j} b_{ij} X_i X_j \qquad \text{with } i = 1\text{-}6 \text{ and } j = 2\text{-}6 \qquad (3.1)$$

where Y = response, X_1 = starch concentration, X_2 = reaction time, X_3 = temperature, X_4 = the amount of NaOH, X_5 = the amount of Na_2SO_4, X_6 = the amount of AGE, b_0 = intercept and b_i = corresponding regression coefficients.

Significant factors were selected based on their F and p values in the statistical analysis. In this study, factors with a p value lower than 0.05 are significant. This means that, for example, when temperature appears to be a significant factor, the obtained DS is with a 95% certainty due to the increased temperature in the temperature range of 20°C to 48°C. This increase is higher than the standard error in DS. For both starches the model could not be optimized further.

Based on these statistical analyses, it was possible to determine the significant process factors. An overview of the effect of the critical process factor is shown in Table 3.3. In general, the temperature has the highest effect on AGE substitution for both starches.

WMS: The statistical reports show that only the effect of the temperature has a pronounced effect on the DS. The effect of other two process factors on the DS, the amount of AGE and NaOH concentration, is certainly present, although both main effects are non-significant. Furthermore, the starch concentration, the reaction time and the amount of Na_2SO_4 appear to have little or no effect. There are no interactions between the main factors which affect the substitution of WMS.

MS: The incorporation of AGE into MS is significantly affected by the reaction time, the temperature and the NaOH concentration. The other three process factors have a very small effect on the substitution. Additionally, some significant effects of interaction between the main factors on the DS were obtained. The combination of reaction time and temperature significantly affects the DS value of MS. Similarly, the combination of the NaOH concentration and the starch concentration has a significant effect on the DS value.

Table 3.3. Effect of the critical process factors in the screening phase for DS_{WMS} and DS_{MS}.

	DS_{WMS}		DS_{MS}	
Factor	F-value	p-value [a]	F-value	p-value [a]
C_{starch} [b]	0.63	0.4340	0.36	0.5520
Reaction time	1.29	0.2674	77.81	**< 0.0001**
Temperature	19.65	**0.0002**	120.02	**< 0.0001**
NaOH [c]	4.07	0.0544	6.71	**0.0164**
Na_2SO_4 [c]	0.81	0.3762	0.58	0.4559
AGE [cd]	4.05	0.0550	1.05	0.3153
C_{starch} * C_{starch}	15.48	**0.0006**	-	-
C_{starch}* NaOH	-	-	4.35	**0.0483**
Reaction time * Temperature	-	-	68.65	**< 0.0001**

[a] Factors in bold are significant ($p < 0.05$). [b] Starch suspension. [c] Process factors are in amounts. [d] Allyl glycidyl ether.

Based on this screening it was not possible to present a mathematical model (= equation) to describe the DS as a function of factor settings, since variables were only set at their min-max levels plus center points. However, this initial screening design allowed a primary selection of the reaction parameters that are significant for the DS. For the final optimization model the reproducibility of the model will be investigated in more detail. There will be even more replicates of the center points and duplicates of all selected star points.

3.3.2 Improving phase

Based on these screening results, our model was improved via a Central Composite experimental Design (CCD). In this improving phase, four variables were evaluated, namely the reaction time, the temperature, the NaOH concentration and the amount of AGE. The levels for the other 2 process factors were constant at: 20% (w/w) Na_2SO_4 on dry starch (ds) and a starch concentration of 300 g kg^{-1} starch suspension. The experimental design scheme contained 25 additional experiments (exp. 36-60, see Table 3.2). The center points have mid-spec factor settings. Experiments at center point conditions have been repeated three times. Also, the influence of the factors at star point conditions has been checked. These star points have factor settings outside of the boundary ranges as defined in the screening phase. Extra samples, with factor settings

within the boundaries, have been performed to improve the predictive power of the mathematical equations.

As can be seen, all additional experiments could be performed on WMS and MS under the chosen conditions, since no gelatinization occurred. The DS values of the experiment 36-60 are up to DS of 0.050 which is lower than those of experiments 1-35 (up to DS of 0.189).

In any experiment, variability arising from a nuisance factor can affect the results, e.g. raw material, equipment, people and time.[12,13] The nuisance factors can systematically controlled through blocking in the statistical analysis. Blocking is advantageous when there is a know factor that may influence the experimental result, but it is not controllable. In our case, it may be caused by the use of different starch batches. Using "blocks" in the statistical evaluation, the analysis of the experiments will focus on the effect of varying levels of the chosen parameters within each block of the experiments. Therefore, these "blocks" are used to level off the differences in DS values of the screening phase and improving phase for both starches using the center points and repetitions of the blocks (Figure 3.1). In the statistical analysis, the DS values of the screening phase will be

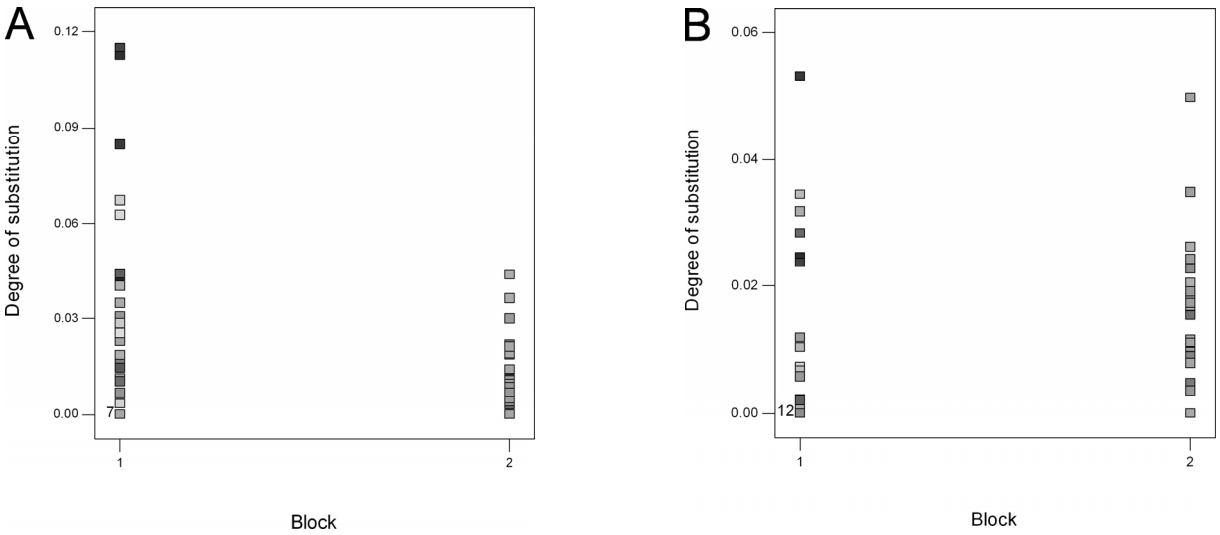


Figure 3.1. Degree of substitution (DS) as function of blocks in which Block 1 and 2 are respectively, the screening phase and the improving phase for WMS (A, DS: 0.00 – 0.12) and MS (B, DS: 0.00-0.05).[i]

[i] The outcome of a substitution reaction is depending on the starch source, reaction conditions, type and extent of the substitution. Any differences in these factors can give variation in the substitution. This is why the results presented here are different from those presented in Chapter 2. This is mainly due to the different reaction conditions and equipment applied here.

leveled off downwards and those of the improving phase will be leveled off upwards. In this way, the blocking effect will normalize the obtained DS values of the two phases. The software will give an overall mathematical equation but will also give the equations for both batches separately.

Furthermore, the substitution of WMS and MS are different under the chosen experimental conditions in both phases. As can be seen in Figure 3.1, WMS was substituted to higher DS than MS. In the screening and improving phase, similar product yields were obtained.

Since reactions were performed in parallel, in a 12-tubes carousel, the error that might occur due to the position of each reaction tube on the circumference of the magnetic plate (i.e. variation in stirring speed, temperature, and turbulence). 24 Experiments were performed at center point conditions with dent maize starch, i.e. all reactions conditions were carried out at 45°C for 10 h with 0.7% ds NaOH and 0.0091% ds AGE. The influence of the position of the reaction tubes on DS was very small: average DS is 0.051 with a pooled standard deviation of 0.0032.

Again, the responses of the different samples were analyzed using the statistical module of Design Expert 7.1.3. The responses are statistically described with second-order interactions and cubic terms, which are also characteristic for an optimization study as CCD. The generalized regression model is shown in equation (3.2).

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{iii} X_i^3 + \sum \sum_{i<j} b_{ij} X_i X_j + \sum \sum_{i<j<k} \sum b_{ijk} X_i X_j X_k$$

with $i = A\text{-}D$, $j = B\text{-}D$, $k = C\text{-}D$ (3.2)

where Y = response, X_A = reaction time, X_B = temperature, X_C = the amount of NaOH, X_D = the amount of AGE, b_0 = intercept and b_i = corresponding regression coefficients. Significant factors were selected based on their F and p values in the statistical analysis. In this study, factors with a p value lower than 0.05 are significant.

The results of the response surface model fitting in the statistical analysis are given in Table 3.4. The model is significant, as is evident from the probability for WMS and MS.

Table 3.4. Regression equation coefficients [a] of a polynomial model[b] for DS on WMS and MS (DS_{starch}).

Coefficient	DS_{WMS}	DS_{MS}
b_0	-0.59044***	-0.10711***
linear		
b_A	0.014647*	0.000539**
b_B	0.040043***	0.005720***
b_C	0.016657***	0.015275***
b_D	0.458410**	3.418310
cross product		
b_{AB}	-0.001110***	
quadratic		
b_{BB}	-0.000591***	-0.000077**
b_{DD}		-172.717***
cubic		
b_{ABB}	0.000018**	
Probability of F	< 0.0001	< 0.0001
R^2 [c]	0.8294	0.6075
Adjusted R^2	0.8029	0.5540
Predicted R^2	0.7336	0.3824
Adequate precision	23.0430	12.5840

[a] *, **, and *** indicates significance at $p < 0.10$, 0.05 and 0.01, respectively. [b] The model for degree of substitution (DS) as a function of the four process factors: *A* (reaction time), *B* (temperature), *C* (the amount of NaOH) and *D* (the amount of allyl glycidyl ether) were calculated as shown in equation (3.2). [c] Determination coefficient (R^2).

The goodness of the fit of the model was checked by the coefficient of determination (R^2). In this case, the value of R^2 for WMS and MS (Table 3.4) indicates that less than 17.1% and 39.1% of the data, respectively, were not explained by the model. The value of adjusted determination coefficients (adj. R^2) are high for significances of the model. Consequently, the predicted determination coefficients are in reasonable agreement with adj. R^2 of both starches and the adequate precisions which measure the signal to noise ratio are greater than 4 (desirable). Based on the improving phase, it is possible to present a final optimization model to describe physical properties as a function of factor settings (Table 3.4). In addition, it was possible to determine the significant process factors.

The main effects of the process factors are shown in the perturbation plots (Figure 3.2). These plots represent the effects of all the factors at a particular point in the design, in this

case the center point scaled at levels. Temperature has the highest effect on the AGE substitution for WMS and MS as obtained in the screening phase.

WMS: The temperature has a highly significant positive effect on the DS. The polynomial suggests that in the temperature range of 20°C to 48°C there is an optimum temperature for the incorporation of AGE (Figure 3.2A). Less prominent, but significant is the NaOH concentration which has also a highly positive effect on DS for WMS. With an increasing amount of NaOH, more AGE is incorporated. Similarly, an increased amount of AGE induces an increased DS for WMS. The reaction time has the lowest effect and is not significant for the synthesis of AHP-WMS. However, a longer reaction time decreases the AGE substitution. It is not with 95% certainty that this difference in DS is due to the process factor reaction time. Furthermore, WMS is easier to substitute than MS, since a higher DS is obtained for WMS.

MS: The temperature has a highly significant positive effect on the synthesis of AHP-MS. Apparently, when the temperature is changed from 20°C to 48°C, there is an optimum temperature for the incorporation of AGE. Also, the NaOH concentration has a positive effect on the substitution. Although AGE is not significant as a main effect for substitution of MS, it is highly significant via the quadratic term as shown in the perturbation plot (Figure 3.2B). Finally, the reaction time is positively correlated to the incorporation of AGE in MS.

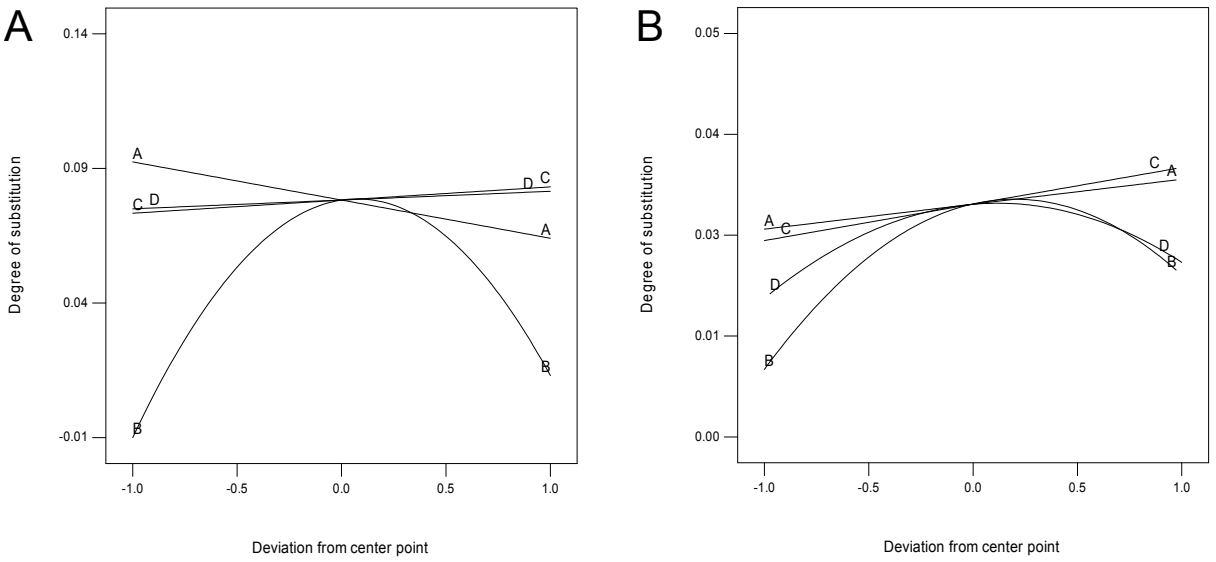
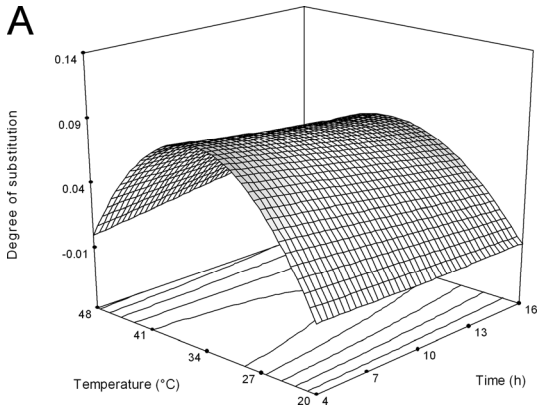


Figure 3,2. Perturbation plots of modified WMS (A) and MS (B); *A*: reaction time, *B*: temperature, *C*: the amount of NaOH and *D*: the amount of allyl glycidyl ether generated at the center point (*A*: 10h, *B*: 34°C, *C*: 0.7% based on dry starch (ds) and *D*: 0.0091% ds).

Waxy maize starch



Dent maize starch

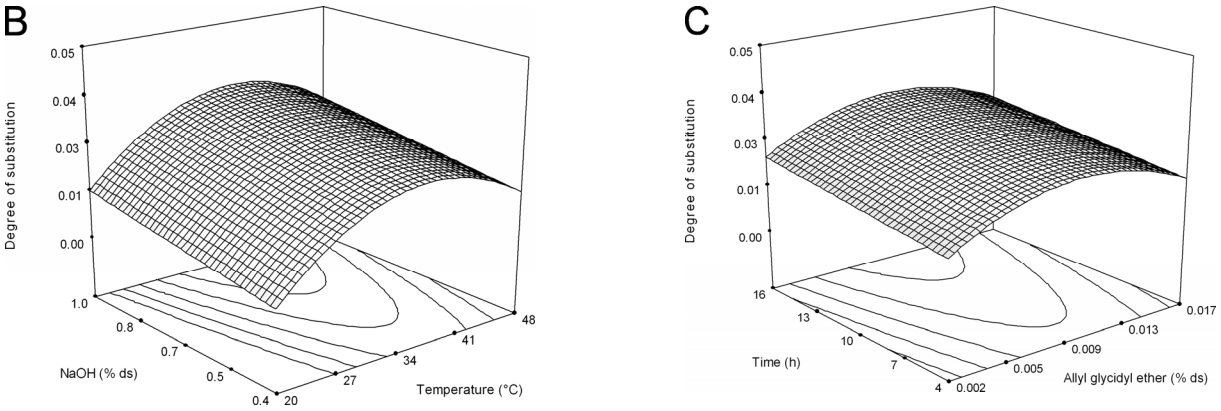


Figure 3.3. Effects of temperature, the amount of allyl glycidyl ether, the amount of NaOH and reaction time on degree of substitution (DS) for waxy maize starch A) and dent maize starch (B and C).

Figure 3.3 shows the effect of the interactions between the process factors on the DS of WMS and MS.

WMS: As shown in the response surface plots for WMS (Figure 3.3A), if the temperature is increased to around 34°C, the highest substitution is obtained. A higher temperature reduces the DS value for the used amounts of AGE or the used reaction time. Furthermore, when the NaOH concentration or the amount of AGE is increased, the DS increases in the temperature range of 20°C to 48°C (not shown).

MS: The response surface plots for MS (Fig. 3.3B and 3.3C) show that when the NaOH concentration is increased at the optimum temperature of 37°C, the highest DS value is obtained. Similarly, the amount of AGE induces the best AGE substitution at 0.01%, and with an increasing NaOH concentration, an increasing DS value is generated (not shown).

3.3.3 Optimization

In the improving phase the most pronounced process factors were obtained. Based on these parameters, the optimized conditions were generated using the optimization module of the experimental design program (Table 3.5). The aim was to have either a maximum DS, or maximum DS at low cost (i.e. lowest amount of AGE) or shortest reaction time for both starches. As can be observed from Table 3.5, the optimal conditions for the best conversion of WMS and MS are different for all optimizations. The optimized conditions generated with a maximum DS with no constraints of the other parameters show that the maximum DS that can be achieved for WMS and MS differs significantly, i.e. 0.102 for WMS and 0.039 for MS. It suggests that WMS is easier to substitute than MS with the generated process conditions. The most important differences in the process conditions between WMS and MS are the temperature and AGE concentration, 34.2°C and 0.0166% ds and 37.0°C and 0.0098% ds, respectively. However, even at these most optimal conditions the conversion efficiency is very low.

Subsequently, reaction conditions were generated which would give the maximum DS at the lowest amount of AGE and no constraints of the other conditions. The best substitution of WMS and MS is again at different temperature, respectively at 34.2°C for WMS and at 37.0°C in case of MS. The maximum DS of AHP-WMS is generated with lowest possible amount of AGE (0.0016% ds) with a remarkably efficient conversion. Oppositely, the synthesis of AHP-MS needs a higher amount of AGE (0.0031% ds) to generate the maximum DS. A slight decrease in substitution of both starches is revealed at these optimized conditions.

Table 3.5. Optimum conditions for the best conversion WMS and MS with a maximum degree of substitution (DS), the lowest amount of allyl glycidyl ether (AGE) and the shortest reaction time.

	WMS			MS		
Process factors	Max. DS [a]	Lowest AGE [b]	Shortest time [c]	Max. DS [a]	Lowest AGE [b]	Shortest time [c]
Reaction time (h)	4.0	4.0	4.0	16.0	16.0	4.0
Temperature (°C)	34.2	34.2	34.3	37.0	37.0	37.0
NaOH [d]	0.99	0.99	0.99	0.99	0.99	0.99
AGE [d]	0.0166	0.0016	0.0016	0.0099	0.0031	0.0038
DS	0.102	0.096	0.096	0.039	0.031	0.027
Conversion eff.[e] (%)	4.3	42.1	42.0	2.8	7.1	4.9

[a] No constraints of the process factors. [b] Maximum DS and no constraints of the other factors. [c] Maximum DS and no constraints for temperature and the amount of NaOH. Variables are calculated as [d] % (w/w) on dry starch. [e] Efficiency.

In the third optimization, the conditions were optimized for a maximum DS at the lowest amount of AGE in the shortest reaction time and no constraints for the temperature and the amount of NaOH. The best results for both starches are achieved after four hours. Even no differences in process conditions are obtained for a maximum DS of WMS because the shortest reaction time has already been achieved in the previous optimization. Consequently, the maximum DS for WMS is the same as in the optimized analysis. The maximum DS for MS (0.027) is lower than the DS value of the previous optimization. The optimum temperature for the synthesis of AHP-MS is 37.0°C. Clearly, in all optimized analyses the best substitution of WMS is at a moderate temperature, around 34°C in all cases. Furthermore, the highest NaOH-concentration is needed for the best conversion of WMS and MS in the generated optimizations. The AHP-MS synthesis needs a higher amount of AGE (0.0038%) than AHP-WMS synthesis in this optimized analysis. Additionally, the conversion for MS is less efficient due to high amount of AGE and very low DS.

3.4 Conclusions

The influence of the different process conditions on the DS of AHP-WMS and AHP-MS is investigated via an experimental design approach. In a two stage approach, a screening phase and an improving phase, the optimized process conditions have been investigated. The statistical analysis shows that the temperature has the largest effect on the modification of both starches. The NaOH concentration and the amount of AGE also have a large but less pronounced effect on the synthesis of AHP-starch. The optimized analysis shows that WMS can be substituted up to DS = 0.102 using 0.0166% AGE based on dry starch (ds), 1.0% ds NaOH at 34°C in 4 hours. Similarly, best conversion of MS can be up to DS = 0.039 using 0.0099% ds AGE, 1.0% ds NaOH at 37°C in 16 h. The optimal generated conditions for the synthesis of AHP-WMS and AHP-MS differ extremely in the maximum DS and the conversion efficiency due to differences in the amount of AGE needed for the synthesis and the accessibility of starch for AGE.

Acknowledgments

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Chapter 4

Physicochemical properties of etherified maize starches

Abstract

The changes in starch properties due to etherification with allyl glycidyl ether (AGE) have been investigated. After etherification of three different maize starches (containing 0.9%, 27% and 70% amylose based on dry starch), no appreciable differences in granular appearance were observed, but the granule crystallinity of these starches was changed. Furthermore, the incorporation of AGE in the starch significantly affects its physicochemical properties: the gelatinization temperatures were decreased and the pasting properties were altered. Both the swelling power and the solubility index increased as the degree of substitution increased. The rheology behavior of the droplets of swollen granules suspension was studied under shear flow conditions.

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4.1 Introduction

Starch and its derivatives are of interest for food and non-food applications e.g. in paper, textile and pharmaceuticals. However, the uses of starch are often limited by unfavorable properties, such as low solubility in water, the tendency of retrogradation etc., depending upon the application.[1] To extend their applications, functional groups can be introduced into starches by a number of chemical or physical modifications in order to provide starches with improved or specific properties. Modified starches generally have markedly altered physicochemical properties, compared to their native starches, depending on the type of functional groups and the degree of substitution introduced.[1] Starch ethers, i.e. hydroxypropylated,[2] allylated[3-8] and methylallylated starches,[4] comprise a wide range of industrial products of different degree of substitution and useful physicochemical properties. For example, the introduction of the hydroxypropyl group in maize starches weakens the bond strength between starch molecules and thereby increases the swelling power and the solubility of the starch granule upon heating.[2]

In previous studies,[5,7] it was found that after air exposure allylated starch exhibited a gummy texture, coated with hard insoluble material, due to cross-linking polymerization caused by oxidation. These properties afford possibilities for application in protective and decorative coatings for wood, glass, metal and other surfaces, for coating and impregnating paper and textiles, and for the preparation of laminated products or rigid plastics. Alternatively, hydrogels could be prepared by allyl starch derivatives copolymerized with methacrylic acid itself and/or its combination with acrylamide. These starch-based hydrogels appeared to have high water absorption capacity, enhanced biodegradability, improved bioadhesive properties, and faster drug release.[3,9] Other graft allylated polymers are used as additives in paper making and for thermoplastic and biocompatible materials.[6,8]

In previous chapters, we reported on a new synthetic route towards 1-allyloxy-2-hydroxy-propyl-starch (AHP-starch) using allyl glycidyl ether under mild reaction conditions.[10] Using chemical characterization methods, differences in molecular structures of the modified and native starch were obtained. However, very little information is available on the granular structure, physical and chemical properties of these kinds of modified maize starches. The aim of this chapter is to study the effect of AHP groups on waxy, dent and

amylose-enriched maize starch, which have reacted with allyl glycidyl ether under mildly alkaline conditions. The thermal and pasting properties, swelling power and solubility index, granule morphology, and flow behavior under shear are studied.

4.2 Experimental section

4.2.1 Starch samples

Amylose-enriched maize starch (70% w/w amylose, AEMS), dent maize starch (27% w/w amylose, MS) and waxy maize starch (0.9% w/w amylose, WMS) were purchased from Tate & Lyle (The Netherlands). Allyl glycidyl ether (AGE) (≥ 99%; cat. A32608) was obtained from Sigma-Aldrich Chemie B.V. (the Netherlands). Polydimethylsiloxane Rhodorsil 47V 200 000 (PDMS) with viscosity of 220 Pa s was provided by Rhodia, France.

4.2.2 Synthesis of AHP-starch

1-Allyloxy-2-hydroxypropyl-starches (AHP-starch) were prepared according to the method described in Chapter 2 with slight modifications. A range of different degrees of substitution (DS) were obtained by adjusting the reaction conditions as follows: Starch was suspended in distilled water to a slurry of 200 or 400 g kg^{-1}. NaOH (2 or 4 % mol mol^{-1} dry starch) and Na_2SO_4 (5 or 35 % g g^{-1} dry starch) were added to the mixture which was heated to 48°C, after which allyl glycidyl ether (0.0016 or 0.0166 % g g^{-1} dry starch) was added. The products were isolated as previously described.[10] The degree of substitution of AHP-starches was determined by ^{1}H NMR using the equation (2.1). A Karl Fisher titration is used to determine the moisture content of the starch compounds as described in Chapter 2.[10]

4.2.3 Scanning electron microscopy

Scanning electron microscopy (SEM) micrographs were obtained on a JEOL JSM-6300 scanning electron microscope. The samples were attached to circular silver stubs with double-sided tape on a carbon sticky tape and coated by platinum sputtering using a Jeol JFC-1200 fine coater (Tokyo, Japan). 'Air-dry' samples were viewed by scanning the total specimen and a representative area was photographed at a magnification up to 6000 ×.

4.2.4 X-Ray diffraction patterns

The X-ray diffraction (XRD) measurements were performed on a Panalytical X'Pert Pro diffractometer (Panalytical, Almelo, the Netherlands) using nickel-filtered CuKα radiation (tube operating at 40 kV and 40 mA). The data were collected by using an automatic divergence slit (5 mm irradiated length) and a 0.2 mm receiving slit with dry starch under N_2 atmosphere. The scanning regions were collected from 7 - 30° (2θ) in step size of 0.02 (2θ). The degree of crystallinity (DC) of the samples was calculated by comparing the diffraction patterns of the samples with that of an amorphous waxy maize sample.[11,12]

Sample preparation: A standard amorphous waxy maize starch was prepared by heating a 10.7% suspension (w/w) for 1 h at 95°C followed by drying under vacuum conditions at 60°C for 48 h. The dried gelatinized starch sample was then crushed to obtain amorphous starch powder. The X-ray diffraction analyses of this amorphous sample gave a typical broad "amorphous" halo, indicating the absence of crystalline structure in these samples.[12] The other samples are crushed under N_2 atmosphere and kept in a desiccator over KOH pellets for one week. The water content of all samples were adjusted via equilibration in a desiccator for one week over saturated K_2CO_3 salt solution at 20°C, providing a relative humidity (RH) of 44% (a_w = 0.44).[11]

4.2.5 Differential scanning calorimetry

The temperature of gelatinization was measured using a Differential Scanning Calorimeter (DSC) (Perkin Elmer DSC-7, Boston, USA). Starch (8.5 mg) was weighed into a 40-µl capacity stainless steel pan (Mettler-Toledo, Switzerland) and distilled water was added with a microsyringe to achieve a suspension with a starch-water ratio of 1:4 (w/w). The pans were hermetically sealed and allowed to stand for 24 h at room temperature before heating in the DSC.[13] An empty steel pan was used as a reference. Sample pans were heated at a rate of 5 °C min^{-1} from 20°C to 150°C. The PE Pyris – DSC-7 software was used for data handling. Onset temperature (T_o), peak temperature (T_p), completion temperature (T_c) and enthalpy of starch gelatinization (ΔH_{gel}) were calculated as described in literature.[14] ΔH_{gel} was based on the dry material (J g^{-1} dry starch).

4.2.6 Viscosity measurements

The pasting properties of starch samples were determined using a Rapid Visco-Analyser (RVA) (Model RVA-4C, Newport Scientific Pvt. Ltd., Warriewood, Australia). Starch (3.0 g dry base) and a weighed amount of distilled water were mixed and stirred in the aluminum RVA sample canister to make a 10.7% starch suspension (w/w). A programmed heating and cooling cycle was used, where the sample was stirred rapidly at 960 rpm for 10 s and was held at 50°C for 1 min. In the next steps, the shear input was decreased to 160 rpm and held constant. The sample was heated to 95°C in 7.5 min, held at 95°C for 5 min, cooled to 50°C in 8.5 min, and then held at 50°C for 3 min.[2] Triplicate tests were performed in each case. Pasting onset temperature ($T_{pasting}$), peak temperature (T_{peak}), peak viscosity, trough or hot paste viscosity, final or cool paste viscosity, breakdown (peak minus hot paste viscosity), and setback (final minus hot paste viscosity) were recorded or calculated (Chapter 1, Figure 1.3).[15]

4.2.7 Swelling power and solubility index

The swelling power of starches was determined via a method adapted from literature.[16] Starch (0.1 g dry base) was mixed in 2 ml distilled H_2O for 4 h at 60°C. The mixture was centrifuged at 15,000 rpm for 15 min. The supernatant was decanted and the wet starch sediment was weighed. Swelling power is defined as the ratio of the weight of the wet sediment to the initial weight of dry starch. The solvent of the supernatant was evaporated at 100°C for 4 h. The solubility index was determined from the ratio of the weight of the dried supernatant to initial weight of the dry starch. The experiment was repeated three times and the mean values are reported.

4.2.8 Dimension of granules and microscopic observations

The dimension of the granules was obtained using a hot stage microscope Olympus BX60 (Olympus Nederland BV, the Netherlands) connected with a Linkam PE94 heat controller (Linkam scientific instruments, UK) and an EHEIM DB IP44 water bath, at a magnification of 20× and 50×. After the heating process (as used for the swelling power and solubility) and cooling to room temperature, suspensions of unmodified starches and modified starches were put onto object glasses, covered with cover glasses, and observed using the light microscope. Photos were taken using an Olympus D70 camera (Olympus

Nederland BV, The Netherlands) and stored in a PC. Photos were further treated, for improving their image, using the analySIS Image Processing software (Olympus Nederland BV, The Netherlands). With ImageJ,[17] the average area was calculated after converting pixels to μm by means of known reference lengths. For these size measurements, more than 100 granules were used from different photos and samples. The outline of the granules was fainter for the modified starches than unmodified starches.

4.2.9 Rheology

Steady shear experiments were carried out at 20°C using a plate-plate geometry (plate diameter 40 mm, gap of 700 or 1000 μm) on a stress-controlled Bohlin Gemini® rheometer (Malvern, UK) equipped with a Peltier temperature control system. Shear rates were varied from 0.1 to 50 s^{-1}. The suspensions of starch granules swollen to their maximum with 100% volume fraction were prepared taking into account the swelling power of each sample determined previously. Dry starch was mixed with water in such proportions that after the heating/cooling cycle there was no free water around the granules and they were swollen to maximal swelling power. The heating/cooling cycle was as follows: suspensions of MS and WMS and their modified counterparts were first heated to 60°C, kept for 30 min, and then cooled to 20°C. These suspensions were used for performing the rheological and rheo-optical measurements.

4.2.10 Rheo-optics

A transparent counter rotating shear cell[18,19] was used to observe the behavior of a droplet of starch suspension under simple shear (Figure 4.1). A droplet of starch suspension with 100% granules volume fraction was placed in PDMS which was chosen as being inert, transparent, Newtonian (in the interval of shear rates used), sufficiently viscous to exert rather high shear stresses and immiscible with aqueous systems thus allowing good visualization of deforming aqueous droplets.[18,19] Both transparent plates (see Figure 4.1) rotate in opposite directions; data on geometry and flow characteristics are stored. An optical microscope placed under one of the rotating plates allows observations in the plane formed by the flow direction and the vorticity axis. All experiments were recorded by a CCD camera coupled to a time-coding system and linked to a DVD recorder and a monitor. By adjusting the relative velocities of the plates, a selected object can be immobilized in the laboratory framework and its behavior can be monitored.

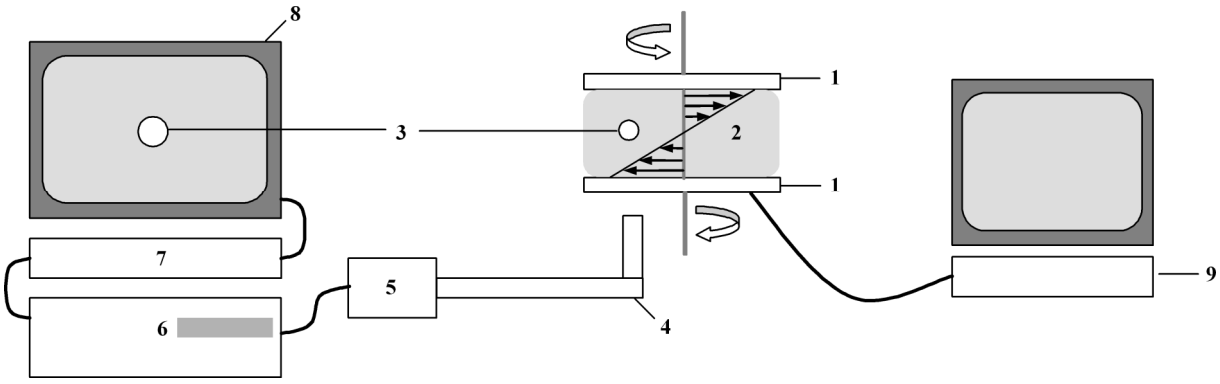

Figure 4.1. Schematic representation of the counter-rotating rheo-optical set-up: transparent plates (1), suspending fluid PDMS (2), droplet of the suspension of swollen starch particle (3), optical microscope (4), CCD camera (5), video recorder (6), time-coding system (7), monitor (8) and computer system (9).

The system PDMS with starch suspension to be placed between the plates was prepared as follows. An adequate volume of polydimethylsiloxane PDMS was placed on the lower plate. A droplet of suspension of swollen starch granules was placed on the top of PDMS and covered with another layer of PDMS. Before setting the gap (900 μm), the system was let to rest for air bubbles to disappear. During each experiment, the system was submitted from low to higher shear stresses by increasing the rotation velocities of the plates. All the experiments were performed at room temperature.

4.3 Results and discussion

4.3.1 Granule morphology of AHP-starch

Three types of starch (WMS, MS and AEMS) were treated with allyl glycidyl ether as described before[10] and their physicochemical properties were compared to the native starches. The granule appearance of the dry starch samples was investigated using scanning electron microscopy (SEM) (Figure 4.2). The appearance of WMS is characterized by its distinct granules, having polygonal shapes, which have irregular or porous surfaces (Figure 4.2A). The MS granules show cubical and more spherical shapes having some smooth and some porous surfaces (Figure 4.2B), while AEMS granules appear as smooth and uniform, with some elongated granules (Figure 4.2C). As in previous studies,[20,21] the shape of granular appearance is more polygonal for amylopectin-rich starches than the amylose-rich starches, but the surfaces of the amylose-rich starches are smoother than those of the amylopectin-rich starches.

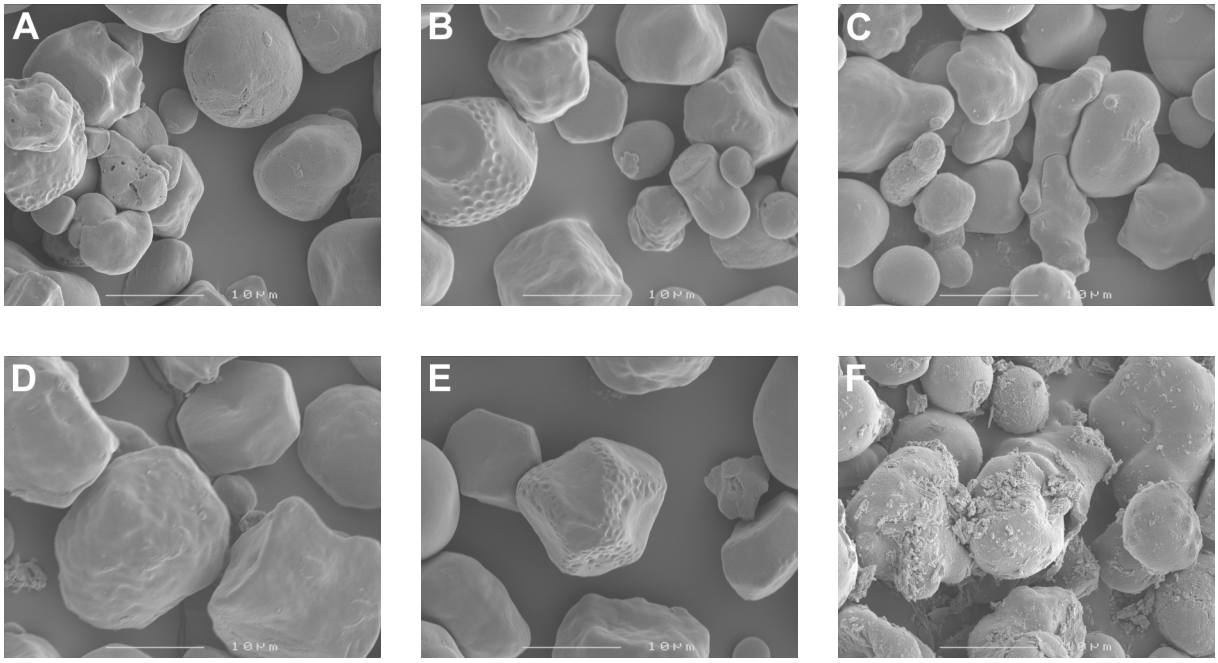

Figure 4.2. SEM-images of (A) WMS (B) MS (C) AEMS (D) AHP-WMS (DS 0.23) (E) AHP-MS (DS 0.11) and (F) AHP-AEMS (DS 0.13). The micrographs (A-F) were taken at 3000 × magnification. Scale bar is 10 μm.

The granules of AHP-WMS, AHP-MS and AHP-AEMS show similar size and appearance. (Figure 4.2D, E and F). However, comparing the micrographs at 6000 × (not shown) shows that the surface of the granule from AHP-WMS and AHP-MS is rougher and more porous as compared to native WMS and MS. The SEM image of AHP-AEMS show smooth granules with small amount of exudates around them. Leaching of amylose at lower temperature after AHP substitution might produce this appearance of exudates. It is reported that leaching of a small amount of exudates occurred at 55°C for high amylose starch.[21]

The results obtained suggest that chemical etherification of WMS, MS and AEMS does not induce significant changes in the shape of the starch granules. Only minor surface modification may occur upon reaction, resulting in a slightly rougher and more porous surface, and a small amount of exudates.

4.3.2 X-diffraction pattern of modified maize starches

Using X-ray diffraction, information on the starch granule crystallinity was obtained. The X-ray diffraction patterns and crystallinity of waxy, dent and high-amylose maize starch

show that the degree of crystallinity (DC) decreases with the increase in amylose content (Figure 4.3 and Table 4.1) which is in agreement with reported data.[22,23] WMS and MS show a typical A-type pattern with strong reflections at 2θ of about 15°, 17°, 18° and 23°. The X-ray diffraction pattern of AEMS has a typical B-type with a strong peak around 17° and small peaks at 20°, 22° and 23°. The additional effect of the substitution on the starch crystallinity is shown in Figure 4.3 and Table 4.1. AHP-WMS and AHP-MS starch show similar X-ray diffractograms as their native form. However, a significant decrease of DC was observed, namely 12.4% for AHP-WMS and 16.8% for AHP-MS, respectively. There was no significant difference in DC between native AEMS and AHP-AEMS. However, their X-ray diffraction patterns are strikingly different from each other. The X-ray diffraction pattern of AHP-AEMS has two decreased peaks at 17° and at ~ 22°, and all peaks shifted to higher 2θ. This observation might indicate that the molecular structure in AHP-AEMS is changed or disappeared, with subsequent rearrangement to another ordered crystal structure that is close to V-type.

In previous studies,[24,25] modified starch with a high level (DS > 0.1) of cationic groups showed also a significant decreased crystallinity. However, starch substituted with a low level of cationization or acetylation (DS < 0.1) revealed no substantial changes in the X-ray diffraction pattern and crystal structures.

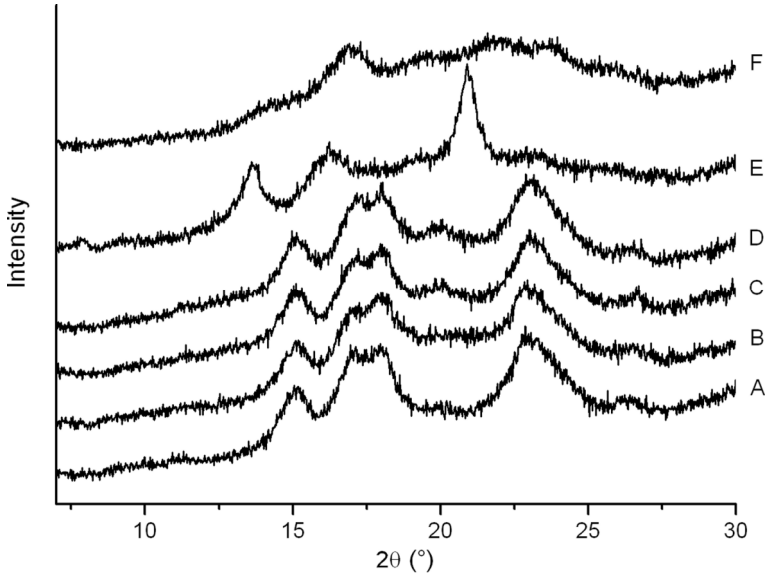

Figure 4.3. X-ray diffractograms of (A) WMS; (B) AHP-WMS (DS = 0.23); (C) MS; (D) AHP-MS (0.11); (E) AEMS and (F) AHP-AEMS (DS = 0.13).

Table 4.1. X-ray diffraction data of native and AHP-starches.

Sample	DS	2θ values (˚ angle)						DC [a] (%)
		23˚	22˚	20˚	18˚	17˚	15˚	
WMS		22.84		20.06	18.18	17.07	15.16	41.9
AHP-WMS	0.23	22.84		20.04	17.92	17.12	15.15	29.5
MS		23.06		20.00	18.00	17.18	15.15	38.9
AHP-MS	0.11	23.02		19.95	18.00	17.23	15.12	22.1
AEMS		23.11	20.91	19.09		16.25		17.5
AHP-AEMS	0.13	23.64	21.95	19.49		16.92		21.5

[a] Degree of crystallinity.

Summarizing, with AHP substitution, no substantial differences in X-ray diffraction pattern for WMS and MS is obtained. The crystallinity level is changed, although the shapes of starch granules remain almost unaltered.

4.3.3 Thermal properties

The DSC thermograms obtained during heating of different aqueous maize starch dispersions are presented in Figure 4.4. The thermograms of all compounds show the endothermic transitions, which are typical for starch. Enthalpy is the latent heat absorbed

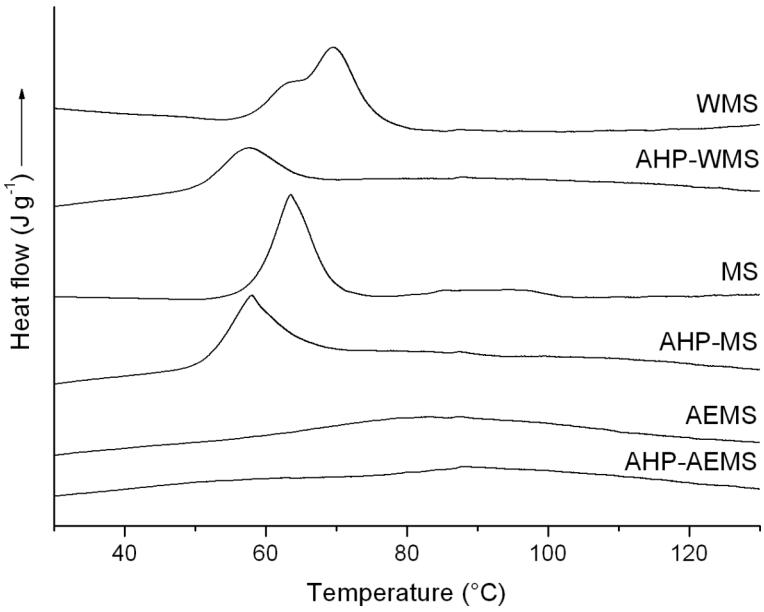

Figure 4.4. Differential Scanning Calorimetry thermograms of native and AHP-starches: WMS, AHP-WMS (DS = 0.23), MS, AHP-MS (DS = 0.11), AEMS and AHP-AEMS (DS = 0.13).

Table 4.2. Thermal properties of native and AHP-starches during heating.[a]

Sample	DS	Gelatinization temperature (°C)			ΔH_{gel} (J g^{-1}) [b]
		T_o [c]	T_p [d]	T_c [e]	
WMS		57.0	69.5	75.9	13.1
AHP-WMS	0.23	50.8	57.3	65.9	4.9
MS		58.5	63.5	69.3	8.9
AHP-MS	0.11	51.4	58.1	67.5	7.9
AEMS		72.7	88.2	115.0	4.5
AHP-AEMS	0.13	59.9	83.5	110.8	7.8

[a] Measured in Differential Scanning Calorimeter; [b] ΔH_{gel} = enthalpy of gelatinization; [c] T_o = onset temperature; [d] T_p = peak temperature; [e] T_c = completion temperature.

by melting of crystallites in the granules, it depends on a number of factors like crystallinity, intermolecular bonding, rate of heating of the starch suspension, presence of other chemicals, etc.[26] Usually the low-temperature endothermic transition is ascribed to amylopectin double helix dissociation and the melting of the crystalline lamellae, while the high-temperature transition is mainly attributed to the dissociation of the amylose-lipid complexes.[27,28] This explains the almost complete absence of the second transition for low-amylose containing starch such as WMS. The starch with high amylose contents gelatinizes at a high temperature because of the presence of amylose-lipid complex, but needs a lower enthalpy, and vice-versa, i.e. starch with low amylose content gelatinized at a low temperature and uses a higher enthalpy (Table 4.2).[29]

Besides the differences of amylose contents in starch, all AHP substituted starches show decreased gelatinization temperatures, while AHP-WMS and AHP-MS show a decreased gelatinization enthalpy (Table 4.2). A low ΔH_{gel} suggests a lower percentage of organized arrangements or a lower stability of the crystals.[29] These differences may be ascribed to the influence of AHP group on the interactions between the starch chains, through steric hindrance by the AHP group, change of hydrophilicity of the starch, or interactions of the hydroxyl group of AHP group with starch chains. Decreases in the thermal parameters are consistent with fewer crystals being present after etherification and with a cooperative melting process enhanced by additional swelling.[2,29,30] A decrease was recorded for the transition temperatures and ΔH_{gel} of all AHP substituted starches, except for AHP-AEMS (Table 4.2). ΔH_{gel} has slightly increased for AHP-AEMS, which could indicate the better molecular order within the granule after AHP substitution. It suggests that AHP substitution in AEMS induces a more ordered orientation of the starch

molecules with the granule, although the starch granules are disrupted at slightly lower gelatinization temperatures.

All AHP-starches gelatinize at lower temperature accompanied with a change in ΔH_{gel}. Variations in enthalpy of substituted starches are indications of structural divergence: molecular structure (amylose and amylopectin fine structures, distribution pattern of AHP groups), and composition (amylose-to-amylopectin ratio, DS).[31]

4.3.4 Pasting properties

Pasting viscosity profiles of starches analyzed using a Rapid Visco-Analyser (RVA) are shown in Figure 4.5 and the results are summarized in Table 4.3. Pasting of starch is a phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules.[32,33] The pasting properties of starch are affected by the amylose and lipid contents and by branch chain-length distribution of amylopectin. Amylopectin contributes to swelling of starch granules and pasting, whereas amylose and lipids inhibit the swelling.[32,34,35]

The RVA results show that the onset pasting temperatures ($T_{pasting}$), i.e. the temperature at which a perceptible increase in viscosity occurs,[15,26] of modified and unmodified starches, are higher than the onset gelatinization temperatures (T_o), determined by DSC, i.e. the temperature at which the starch granules gelatinized. ΔT ($T_{pasting}$-T_o) for the native starches is for WMS ≈ 12°C, for MS ≈ 16°C. For AEMS, ΔT could not be determined, while for the AHP substituted WMS, MS and AEMS is respectively ≈ 5°C, ≈ 12°C and ≈ 34°C. Thus, the differences between T_o and $T_{pasting}$

Table 4.3. Pasting properties of native starch and AHP-starches.[a]

Sample	DS	$T_{pasting}$ [b] (°C)	T_{peak} [c](°C)	Viscosity (cP)				
				Peak	Hot paste	Final paste	Breakdown [d]	Setback [e]
WMS		68.5	78.3	3934	1637	1826	2297	189
AHP-WMS	0.23	55.5	68.0	10841	5402	9647	5439	4245
MS		74.9	94.7	3648	2188	3950	1460	1762
AHP-MS	0.11	63.7	80.8	5885	1891	7097	3994	5206
AEMS		-	-	-	-	36	-	36
AHP-AEMS	0.13	94.1	95.0	74	-	64	74	64

[a] Measured in Rapid Visco-Analyser; [b] $T_{pasting}$ = pasting onset temperature; [c] T_{peak} = peak temperature; [d] peak viscosity minus hot paste viscosity; [e] final paste viscosity minus hot paste viscosity

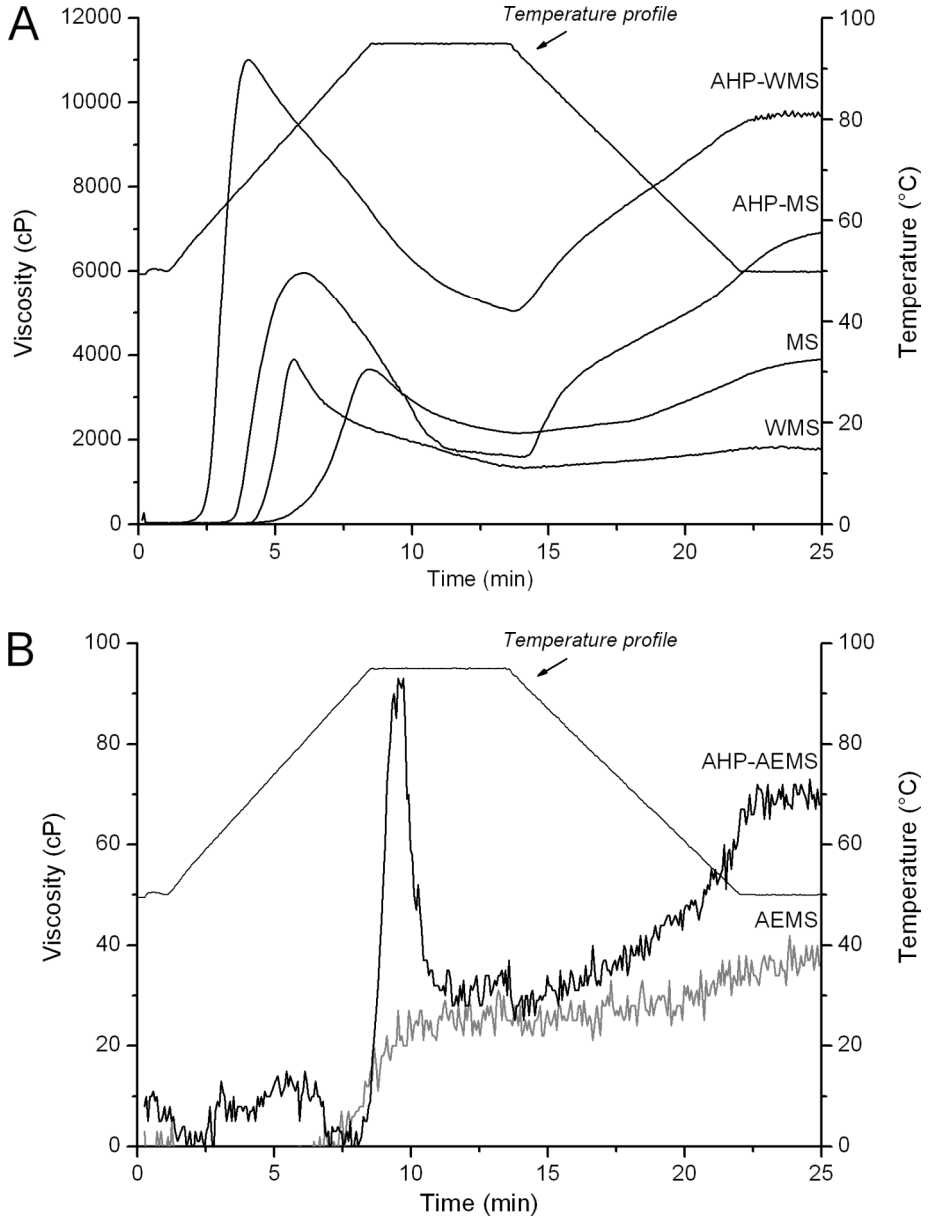

Figure 4.5. RVA pasting profiles of native and AHP-starches: (A) WMS, AHP-WMS (DS = 0.23), MS and AHP-MS (DS = 0.11) (B) AEMS and AHP-AEMS (DS = 0.13).

decreases after substitution. This suggests that the gelatinization and the increase in viscosity occurred at a shorter temperature range for AHP substituted starches than for their non-modified starches.

Furthermore, the shapes of the pasting curves differed markedly after etherification. All AHP-starches results in an increased setback, indicating that retrogradation takes places, i.e. a process in which molecules comprising gelatinized starch starts to reassociate in an ordered structure.[32] It is reported in literature that waxy maize starch has a lower

pasting temperature, a higher peak viscosity, and lower setback than maize starch.[34] In general, the setback reflects the gel network formation involving amylose which is very low for WMS,[36] and that is indeed what we observed (Table 4.3). AHP-WMS show a higher peak viscosity, higher breakdown, higher setback and a higher final paste viscosity than its native starch. A similar but smaller effect has been obtained for the differences in MS and AHP-MS. A large swelling of the granule can lead to an increased peak viscosity.[2] Presumably, the substitution induces less ability to withstand heating and shear stress and, therefore, the breakdown increases. The higher setbacks, which were obtained, suggest that the tendency for recrystallization is increased for AHP-WMS and AHP-MS. Thus, the ability to form a viscous paste is increased when the starches are etherified. The final paste viscosity of the AHP substituted starch is higher than the native starches and that means the materials forms a better gel after cooling than the native starches. These differences may be ascribed to the structural changes of starch granules taking place during the reaction conditions used.[2]

Because of a high amylose content in AEMS, the starch hardly shows any swelling,[36] and thus, displays very low viscosity as shown in Figure 4.5B. The substituted AEMS induces a low peak viscosity, low breakdown and low setback. The gelatinization is initiated at lower temperature, and AHP-AEMS is prone to swelling. The swelling of granules leads to a small peak viscosity. The setback and breakdown are similar to the peak viscosity, which means the formation of gel network did not seem to occur in this modified starch.

4.3.5 Swelling and solubility index versus degree of substitution

The swelling power and solubility index is dependent on the starch species, and on the type and extent of modification.[37] During heating, water penetrates into the more accessible amorphous region of the granule, resulting in hydration and limited swelling. At the gelatinization temperature, the swelling of the amorphous phase (water-penetrated phase) accelerates the disruption of the crystalline region. This development can be ascribed to the swelling power of the starch granules. The swelling power of all AHP-starches increases as substitution increases (Figure 4.6A). AHP-WMS shows the highest increase of swelling power as the DS increases. The lowest increase of swelling power is for AHP-AEMS due to the high amylose content, and its high gelatinization temperatures. The incorporation of allyl glycidyl groups seems to reduce the interactions between

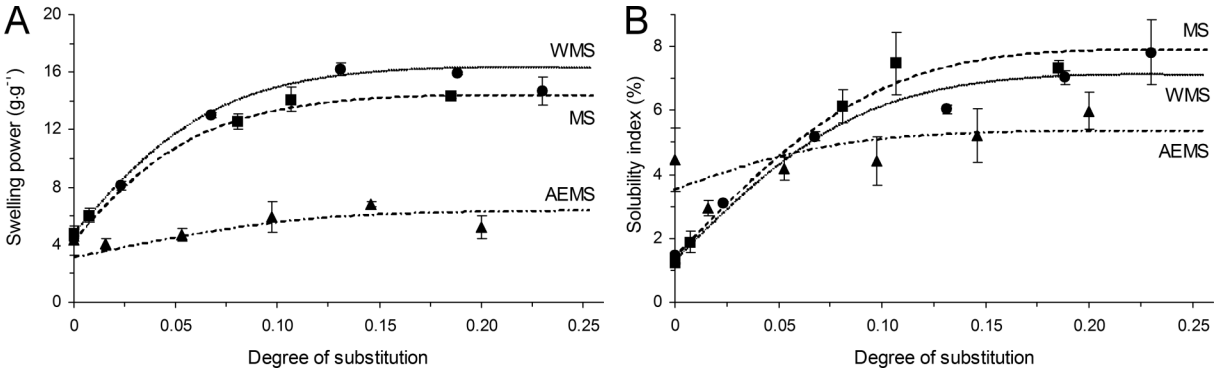

Figure 4.6. Swelling power (A) and solubility index (B) of native and AHP-maize starches at 60°C. The lines are given to guide the eye; dotted, dashed and dashed-dotted lines correspond to modified ● WMS, ■ MS and ▲ AEMS, respectively.

molecules of starch inducing the increase of hydrophilicity of starch, i.e. the incorporation of substitution changes the polymer-solvent interaction causing lower gelatinization temperatures (described by DSC) and increases swelling power. In previous studies on hydroxypropylated,[2] carboxymethylated[38] and acetylated starch,[39] an analogous tendency of increased swelling power with increased substitution is observed.

The solubility index of the native and modified starches was also studied as a function of DS. The solubility is associated with the hydrophilicity and ease of disruption of the starch granules. AHP-WMS and AHP-MS showed an increased solubility upon substitution (Figure 4.6B). Due to the high content of crystalline amylopectin, the modified WMS shows a lower increased solubility for an increasing DS than for AHP-MS. A similar effect for acetylated WMS, MS and AEMS was described in other substituted starch studies.[40,41] AEMS has the lowest increase of the solubility index when the substitution increases, due to its high amylose content and its gelatinization temperatures.

The swelling power and solubility index of all starches seems to go to a saturation limit. This tendency was observed also for other substituted starches.[37-39,41] The question is whether this saturation limit is due to the distribution pattern of AHP groups, or to the degree of substitution. The purpose was to measure the relative swelling capacity and the amount of soluble material for starch granules at a certain temperature. We know from the previous chapters that the amorphous region in the starch granule is more substituted than the crystalline region. Because of the AHP substitution in the amorphous region, greater water uptake is permitted resulting in an increase in the swelling of the granule.

Furthermore, the swelling capacity for the AHP starches, depending on the substitution, could already be high at room temperature compared to the maximum swelling capacity of the native starches. Based on this information, we assume that small differences in swelling capacity for modified starches may be induced at higher temperature, due to the already high swelling capacity at room temperature. A similar suggestion was made in the study to carboxymethylated starch, which had a swelling capacity at room temperature between 60 to 80% of the maximum swelling capacity for the native starch at 95°C.[38] Consequently, a saturation limit for the swelling power and the solubility index for modified starch at a certain temperature might be approached, depending on the substitution. Other properties such as temperature of gelatinization and viscosity of the different starches modified with different residues, related to swelling and solubility of starch granules, could be investigated to elucidate this tendency.

In general, increased DS leads to increased swelling power, which indicates a more rapid hydration of the modified starch granule than its native starch. A higher accessibility of the amorphous regions of the starches contributes to an increase of the solubility index, as is observed for the waxy and dent maize starch derivatives.

4.3.6 Particle dimension of swollen granules

It is known from literature[42] that the granule area increases in the order AEMS-MS-WMS within an certain temperature range. When the starch granule is heated, the growth of starch granules is controlled by two factors: swelling and dissolution. Most of the granules remain intact below their destruction temperature (T_c in DSC measurements, Table 4.2).[34,42] Therefore, the area distribution of all the granules heated at 60°C was studied using a hot stage microscope and the results are illustrated in Figure 4.7 and Table 4.4 for native starches and AHP substituted starches.

The results show that the substitution of three different starches has considerable influence on the area distribution of their swollen granules (Figure 4.7B). The highest swelling and area distribution of swollen granules was observed for AHP substituted WMS containing the highest amount of amylopectin. The mean area of the swollen granules of WMS increased up to nine times upon AHP substitution. In untreated starch, 50% of the granules have a surface area between 100 and 250 μm^2, while about 40% of the granules of AHP-WMS have, after swelling, a mean surface of 750-2500 μm^2.

AHP-WMS swollen granules are distributed over a broad interval of areas ranging from 100 to 5000 μm^2. Since the amorphous region in the granule is more substituted than the crystalline region,[10] this effect might be explained by easier access of the moisture through amorphous regions for AHP-WMS than WMS. Less influence of the AHP substitution on the area of the swollen granules is observed for MS and AEMS starch,

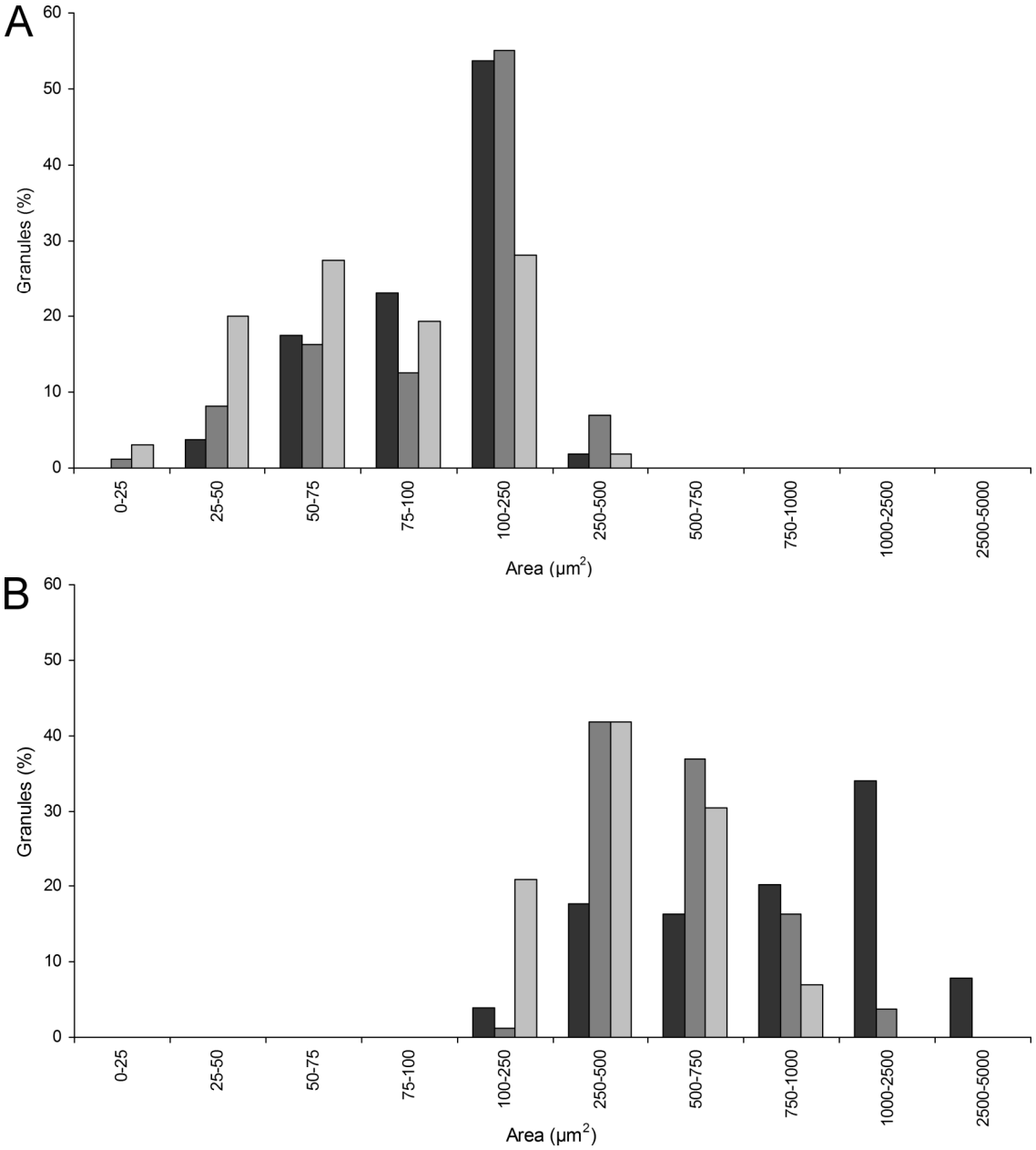

Figure 4.7. Average area distribution of granules heated at 60°C for 4 h in aqueous solutions (a) unmodified ■ WMS ■ MS □ AEMS and (b) modified starches: ■ AHP-WMS (DS 0.19), ■ AHP-MS (DS 0.19) and □ AHP-AEMS (DS 0.17).

Table 4.4. Mean values and standard deviation of swollen starch granules' area heated at 60°C for 4 h of native and AHP substituted starches.

Sample	DS	Mean area (μm^2)	Standard deviation (μm^2)
WMS		120	60
AHP-WMS	0.19	1160	810
MS		130	70
AHP-MS	0.19	570	230
AEMS		90	50
AHP-AEMS	0.17	440	200

respectively. Nonetheless, the mean areas of MS and AHP-MS differs more than a factor four. A shift in area distribution from around 100-250 μm^2 for MS to 250-750 μm^2 for AHP-MS is obtained. Compared to the other native starches tested, AEMS has swollen only slightly at this incubation temperature, due most probably to the high amylose and lipid content that inhibits swelling. Still, the mean area of AEMS swollen granules after AHP substitution has changed considerably, from 25-100 μm^2 for AEMS to 100-500 μm^2 for AHP-AEMS, because of its lower gelatinization temperature (DSC, Table 4.2). Based on these results, we may conclude that AHP substitution induces a higher mean area and broader area distribution of the starch granule by swelling at 60°C for 4 h and the loss or change of crystalline structure in the granule.

4.3.7 Behavior of a droplet of starch suspension under shear conditions: a rheological and rheo-optical study

The goal of this section is to demonstrate a qualitative difference in the behavior of modified and native starch suspensions under flow.

Steady-state flow of starch suspensions

The flow of starch suspensions with 100% granule volume fraction is shown in Figure 4.8. It is clear that the viscosity of non-modified starch is much higher than the one of modified starch. This is due to a low swelling power of native starch granules (Figure 4.6) leading to higher granule rigidity and higher polymer concentration in 100% granule volume fraction suspension. All suspensions behave as non-Newtonian fluids: they follow the power law dependence viscosity ~ (shear rate)$^{-n}$ with larger exponents for non-

modified starch as compared with modified ones. Dent maize starch seems to show a plug flow with the exponent close to one.

Rheo-optics: visual observations

The optical micrographs of the droplets of AHP-WMS and AHP-MS granules suspension at 100% volume fraction swollen at maximum and immersed into PDMS are shown in Figure 4.9. The initial shape of the droplets of swollen granule suspension is more or less spherical (Figure 4.9A and E). Their shape is less regular when the granule is less "soft" because of its decreased swelling power (compare with Figure 4.6). Both suspensions of modified starch behave in a similar way: at low shear stresses (σ) the droplets deform (Figure 4.9B and F). At increasing shear stress (or shear rates, which is the same because PDMS used is a Newtonian fluid in this interval of shear rates) the droplets keep elongating (Figure 4.9B, C, F, and G) before breaking into two or more smaller ones, approximately of the same size (Figure 4.9D and H). During the elongation, the starch suspension moves as a thick paste inside the droplet around the vorticity axis. For the AHP-WMS suspension, rupture occurs at about 500 Pa, for AHP-MS at 1400 Pa. Each newly formed droplet elongates up to breaking under increasing shear. For example,

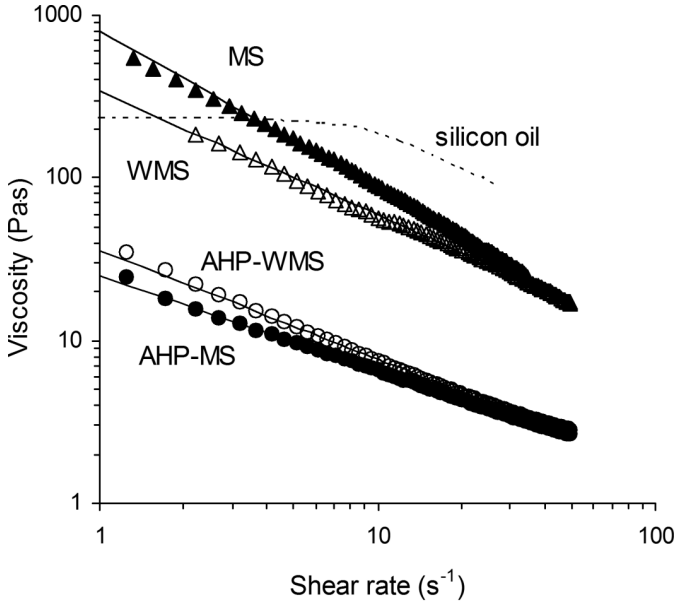

Figure 4.8. Flow curves of modified and non-modified starch suspensions with 100% granule volume fraction. Points are experimental data, lines are power law fits with n = 0.58 (AHP-MS, DS = 0.19), 0.67 (AHP-WMS, DS = 0.19), 0.74 (WMS) and 0.95 (MS). Dashed line corresponds to PDMS.

AHP-WMS underwent another break-up into two main droplets at about 680 Pa (not shown). At a fixed shear stress, the droplet breaks down to a certain minimal radius for which no more break-up occurs. Such behavior is quite similar to the one of a viscous liquid droplet immersed into an immiscible matrix.

Unlike AHP-WMS or AHP-MS, a droplet of a non-modified starch suspension prepared in equivalent conditions (100% volume fraction, maximal swelling) and immersed into PDMS does not have a spherical shape when no shear is applied (see an example of WMS and MS droplets in Figure 4.10). It looks like an agglomerate of rigid particles, which is more or less the case. These granules have a much lower swelling power than the AHP modified starches (Figure 4.6), they are thus more rigid and much smaller (Table 4.4) and polymer concentration inside the granule is much higher, this being responsible for the dark color of the droplet (Figure 4.10). Because of particles rigidity, the interfacial tension is playing a smaller role and the droplet is not adapting the spherical shape. The higher is the swelling power, the softer are the particles and the more circular is the shape of the droplet at rest (compare Figure 4.9A, E and 10A, D, which are ordered with decreasing of swelling power according to Figure 4.6). Under shear, the droplet of WMS suspension deforms but does not elongate like the ones of modified starch. A "tail" can be seen as the droplet rotates (Figure 4.10B and C). Solvent (water) can be seen in-between the granules, helping to keep them together. The droplet of MS suspension only rotates but does not deform.

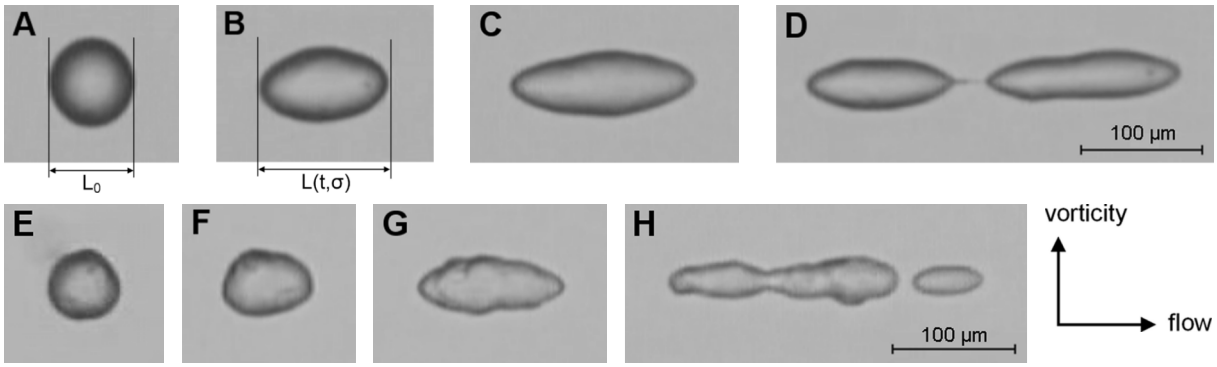

Figure 4.9. Optical micrographs of AHP-WMS (A-D) and AHP-MS (E-H) droplets of suspensions of 100% granules volume fraction, deforming in time (t) and under shear stress (σ). AHP-WMS: 0 Pa, initial droplet diameter $L_{\text{AHP-WMS, 0}}$ = 80 µm (A); t = 35 s, 300 Pa (B); t = 54 s, 420 Pa (C) and t = 67.5 s, 510 Pa (D); AHP-MS: 0 Pa, initial droplet diameter: $L_{\text{AHP-MS, 0}}$ = 70 µm (E); t = 50 s, 710 Pa (F); t =70 s, 1070 Pa (G) and t =90 s, 1370 Pa (H). The gap was 900 µm. Flow and vorticity direction in the rotating cell are shown by arrows.

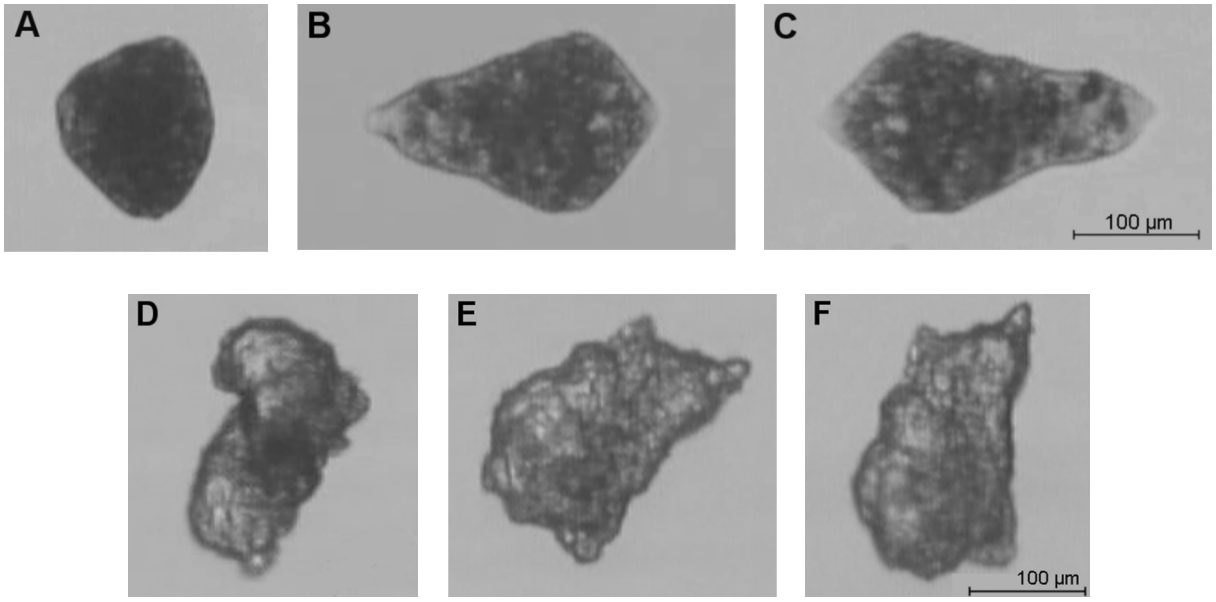

Figure 4.10. Optical micrographs of a droplet of WMS and MS suspensions with 100% volume fraction granules, at different shear stresses: WMS at 0 Pa, initial size: $L_{WMS,\,0}$ = 145 µm (A); 650 Pa (B) and 1090 Pa (C); the "tail" is visible on the left (B) and right (C) of the picture showing the rotation of the droplet; MS at 0 Pa (D); 830 Pa (E) and 1300 Pa (F). The gap was 900 µm.

Deformation of droplets: comparison between modified and non-modified starches

An example of the evolution of the droplet relative length $L(t, \sigma)/L_0$ (Figure 4.9) as a function of strain (shear rate × time, s s^{-1}) for AHP-WMS suspension is presented in Figure 4.11. As was shown in Figure 4.9, the droplet elongates up to rather high values, and at a certain strain (≈ 140 s.u.) rupture occurs which is reflected by a sharp decrease of L/L_0. The evolution of one of the "secondary" droplet is then followed as a function of strain and a second rupture occurs around 330 strain units or 680 Pa.

The comparison of the evolution of L/L_0, up to the first break-up, for AHP-WMS and AHP-MS starch suspension droplets as a function of strain is shown in Figure 4.12. The rupture of AHP-MS droplets occurs much later, or at higher stresses (1370 Pa), than for AHP-WMS droplet (510 Pa). When making qualitative analogies with the deformation and break-up of a liquid droplet in an immiscible matrix,[43] for the cases when viscosity of the droplet is lower than the one of the suspending fluid, droplet break-up should occur at lower shear stresses for a fluid with a higher viscosity (so-called Grace diagram) keeping all the other system parameters like the interfacial tension, droplet initial size and matrix

viscosity were kept the same. This is what we obtained: both AHP-MS and AHP-WMS suspension viscosities are lower than the one of PDMS (Figure 4.8), whereas AHP-WMS viscosity is slightly higher than the one of AHP-MS (Figure 4.8) in the region of applied stresses and all other parameters are practically the same. However, even fitting with the classical theoretical prediction, such a comparison remains qualitative and rather superficial because the Grace diagram is made for Newtonian emulsions in the steady state, which is not the case of starch suspensions shown in Figure 4.11 and 4.12. Further studies are needed for making quantitative conclusions.

The next step was to compare the deformation of the droplets of modified starch suspensions with their native ones. An example is given in Figure 4.13. Even initially large WMS droplets do not break up and seem to show saturation at deformations around 1.8, which are much lower than the ones recorded for modified starch droplets before their rupture (dashed lines in Figure 4.13). The deformation of the MS droplet is negligible (data not shown in order not to overload the graph), not higher than 10%, which is within experimental errors.

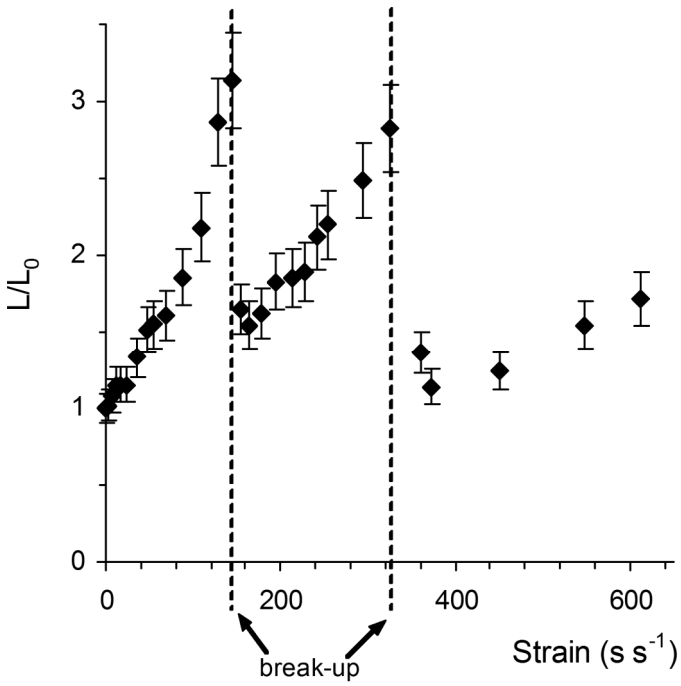

Figure 4.11. Evolution of the relative length of a droplet of AHP-WMS suspension (100% granule volume fraction, maximal granule swelling) as function of strain: droplet initial size was $L_{\text{AHP-WMS, 0}}$ = 80 µm, rupture at 510 and 680 Pa is shown by dashed lines, shear rates were varied from 0 to 4 s^{-1}, shear stresses from 0 to 900 Pa.

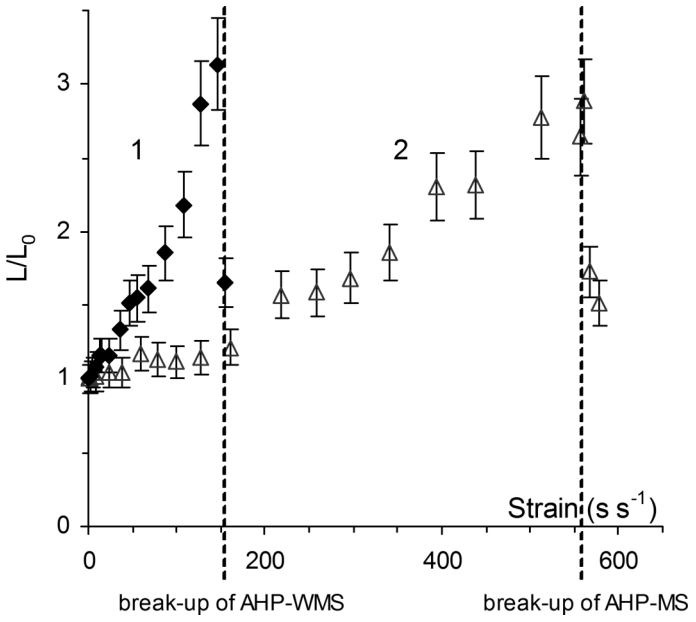

Figure 4.12. Evolution of the relative droplet length as a function of applied strain for AHP-WMS (1) and AHP-MS (2) starch suspension droplets, granule volume fraction 100%, granules swollen at maximum, $L_{\text{AHP-WMS, 0}}$ = 80 µm and $L_{\text{AHP-MS, 0}}$ = 70 µm. The data for AHP-WMS are the same as in Figure 4.11. Shear rates were varied from 0 to 6.5 s^{-1}. The first break-up of AHP-MS droplet occurs at 1370 Pa.

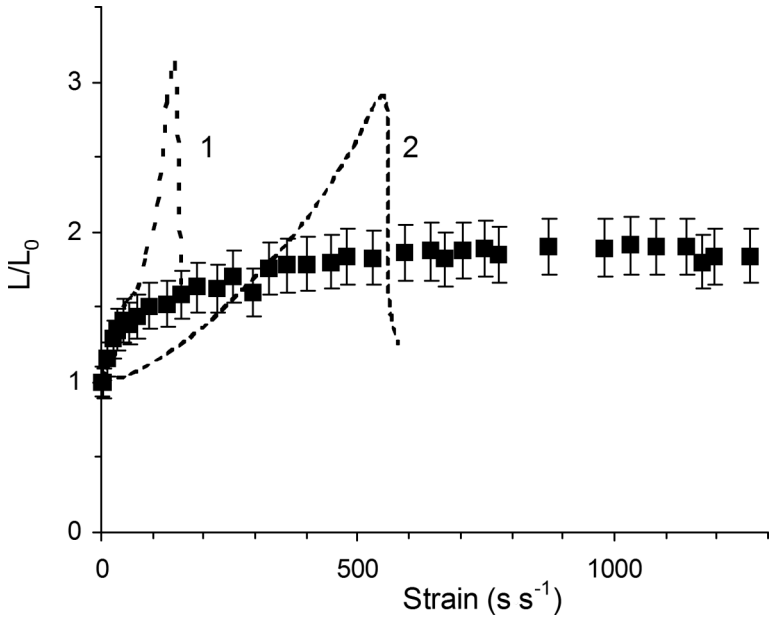

Figure 4.13. Comparison of the evolution of droplet suspension of WMS starch with 100% volume fraction, maximal granule swelling (dark points), as a function of strain, with droplet behavior of AHP-WMS (1) and AHP-MS (2) suspensions before break-up. $L_{\text{WMS, 0}}$ = 145 µm, shear rates were varied up to 8.2 s^{-1}, shear stresses up to 1800 Pa.

Such a different behavior of the droplets of modified and non-modified starch suspensions is related to different swelling power of the granules and thus polymer concentration and granule rigidity, the latter governing suspension viscosity. For the case of WMS suspension, its viscosity is still lower than PDMS (Figure 4.8). When again making analogies with classical liquid droplets suspended in an immiscible matrix, native starch droplets should break-up at lower capillary numbers than the modified starch droplets, because of higher viscosities of the non-modified starch suspensions and larger droplet size.[43] We obtained the opposite result: no break-up was observed for the native starch suspension even at higher stresses and for an initially larger droplet (Figure 4.13). Lower capillary numbers mean lower stress at break-up and/or smaller initial droplet size (which does not correspond to our case, as mentioned above), or higher interfacial tension. The notion of the interfacial tension for a suspension with 100% particle volume fraction is a delicate point. It seems that there is some free liquid in-between the granules (Figure 4.10B and C) even if suspensions were prepared in such a way that the granules should absorb all water. Is this liquid pure water that was left not adsorbed or water or polysaccharide solution released from the granules due to shear? The release of solvent from a swollen synthetic micro-gel particle under shear has been reported.[18,19] If the liquid between the granules is water, the droplet is not breaking because of the high interfacial tension water/PDMS. This could explain the absence of the break-up of the droplet of native starch suspension.

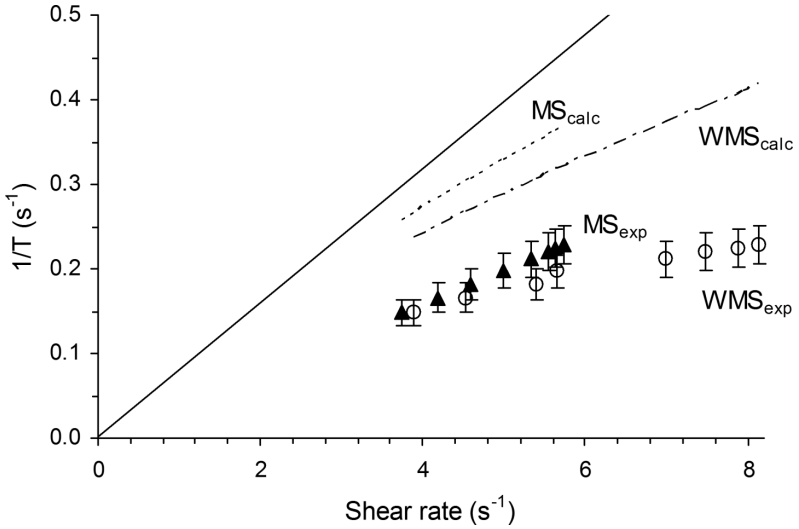

Figure 4.14. Rotation period as a function of shear rate for droplets of WMS (open points) and MS (dark points) suspensions. Solid line corresponds to Jeffery law (eq. 4.1); dashed and dashed-dotted lines correspond to calculated dependences (eq. 4.2) for MS and WMS, respectively.

It was possible to measure the periods of rotation of WMS and MS droplets and compare these with the approaches developed for a rigid particle rotating in a fluid under shear and for a liquid Newtonian droplet. The results are shown in Figure 4.14.

It is known that the rotation period of a rigid spherical particle immersed in a Newtonian fluid is described by Jeffrey's law (4.1),[44] where the period T is inversely proportional to the shear rate γ:

$$T = \frac{4\pi}{\gamma} \tag{4.1}$$

In the case of a sheared droplet, viscous forces exerted by the suspending matrix induce circulation of the fluid inside the droplet,[45] and the mean period of its circulation on the droplet surface is given by expression (4.2):

$$T = \frac{4\pi}{\gamma} \frac{p+1}{\sqrt{p(p+2)}} \tag{4.2}$$

where p is the ratio of droplet to suspending matrix viscosity. The slope of T = f(γ) depends on viscosity ratio: it increases with increase of p and reaches Jeffrey law (4.1) at p = ∞.

All experimental points (Figure 4.14) fall far below the solid line corresponding to the rotation of a solid particle and below the dashed lines that correspond to the period of liquid circulation in a droplet, the latter calculated taking into account the viscosity of each suspension at the corresponding shear rate from Figure 4.8. The fact that experimental data are far from those predicted by equation (4.1) was to be expected: the droplet of non-modified starch suspensions is highly asymmetric and deformable. Asymmetry, inhomogeneity, the non-Newtonian character of suspension viscosity of the droplets studied and their possible visco-elastic behavior can explain the difference between experimental data and liquid droplet approach.

4.4 Conclusions

The observed changes in starch properties due to etherification have been described in this chapter. In dry granular starch, no appreciable changes in granular form for the three different maize starches are obtained. A high degree of substitution with AHP groups causes a large decrease in starch crystallinity for AHP-WMS and AHP-MS. AHP-AEMS seems to have a slightly better DC, which is confirmed by a higher ΔH_{gel}. The incorporation of AHP substituents in the starch molecules significantly affects their physicochemical properties. All gelatinization temperatures are reduced after modification. Furthermore, the altered pasting properties lead to shear thinning during heating and a better gel network after cooling for AHP-WMS and AHP-MS. The swelling power of all starches increases as DS increases. Similarly, the solubility index of WMS and MS increases as DS increases. Microscopic measurements confirm the swelling capacities of the three starches in order of AHP-WMS > AHP-MS > AHP-AEMS at 60°C. Granules with increased swelling power induce a desirable change in the flow behavior under shear, making them interesting for biomaterials.[46] Immersed into PDMS, droplets of AHP modified WMS and MS suspension (100% volume fraction, maximal swelling) break up into several smaller droplets under shear, whereas the native starches do not elongate, but only form a "tail" or only rotate in the vorticity direction.

Acknowledgments

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Chapter 5

Synthesis and characterization of epoxy starch derivatives

Abstract

Epoxy starch derivatives were synthesized by epoxidation of allylated starch. The reaction was performed with low substituted 1-allyloxy-2-hydroxypropyl-waxy maize starch (AHP-WMS; 1.42 mmol allyl groups per gram dry starch) using H_2O_2 and acetonitrile. Via a two step spectrophotometric assay, it was determined that epoxy-WMS contained 0.13 ± 0.03 mmol epoxy groups per gram dry starch. Enzymatic digestibility, swelling capacity and solubility were slightly reduced after epoxidation. The structure of epoxy-WMS was characterized by enzymatic degradation followed by chromatographic and mass spectrometric techniques. The mass distribution of enzyme-resistant oligomers showed significant differences between AHP-WMS and epoxy-WMS. Only a small amount of epoxidized oligomers were found in the enzymatic digested products of epoxy-WMS. Apparently, the epoxidation reaction is highly efficient but subsequent reactions of epoxy groups lead to a considerable amount of cross-links and hydrolysis.

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5.1 Introduction

Because of biocompatibility, degradability and low costs, starch and starch derivatives are used as excipients for tabletting as well as matrices for delivery systems in pharmaceutical industry.[1,2] There is a strong interest in chemically modified starch derivatives possessing functional groups that allow easy binding of ligands to the surface. Amongst these starch products are derivatives possessing epoxy functions.[3-6] These reactive epoxy groups allow binding of ligands such as polyamines, peptides or amino acids. Moreover, epoxy groups can advantageously serve as cross-linker in starch. Hence, these starch derivatives can be used for various purposes, such as carriers for delivery systems and controlled release, but also in coatings and stabilizers.

Incorporation of epoxy groups onto carbohydrates can be performed directly[7-9] and indirectly.[3,10,11] Direct integration of epoxy groups can be achieved using epichlorohydrin[5,7] or diepoxides.[7,8] Disadvantages of these routes are the side reactions during the synthesis, use of hazardous reagents like butadiene diepoxide as well as harmful solvents such as dimethyl sulfoxide and dichloromethane. Indirect incorporation of epoxy groups can be accomplished by oxidation of double bonds attached to the polysaccharide using peracids and/or hydrogen peroxide.[4,10] Allyl cellulose was efficiently epoxidized using peracids, although this reaction pathway resulted in partial hydrolysis of the cellulose derivative. Furthermore, the used solvent (dichloromethane) is not applicable for large scale reactions.[4]

In previous Chapters 2 and 3, the double bonds were introduced into starch by the reaction of maize starch and allyl glycidyl ether (AGE). Using mild reaction conditions, starch granules reacted with a small amount of AGE, and low etherified starch derivatives (degree of substitution up to 0.2) were obtained. These low allyl substitutions already induced alteration in properties and behavior of starch.[12,13] The current chapter presents the preliminary results obtained from the epoxidation of double bonds in these allyl waxy maize starch derivatives. Both the amount of epoxy groups as well as the structure of epoxy starch derivatives were studied.

5.2 Experimental part

5.2.1 Materials

1-allyloxy-2-hydroxypropyl-waxy maize starch (AHP-WMS; DS_{allyl} = 0.23) was manufactured as described before.[13] Acetonitrile (99.5%, cat. 258560010) was purchased from Acros Organics (Belgium). Hydrogen peroxide (35% weight solution, cat. CL002306.1000) was obtained from Chem-Lab NV (Belgium). 1,6-hexanediamide (HD) (≥ 99%, cat. 33000) and 4-(4-nitrobenzyl pyridine) (*p*NBP) (≥ 98%, cat. 73210) were from Fluka (Switzerland). Allyl glycidyl ether (AGE; ≥ 99%, cat. A32608), potassium carbonate (≥ 99%, cat. 367877), phosphate salts, dimethyl sulfoxide (99.5%, cat. 472301), ethylene glycol (≥ 99%, cat. 293237), α-amylase (EC 3.2.1.1) (cat. A4551, from *Bacillus licheniformis*, 408 U mg^{-1}) and amyloglucosidase (EC 3.2.1.3) (cat. A7255, from *Rhizopus* sp., 11600 U g^{-1}) were obtained from Sigma-Aldrich Chemie BV. Pullulanase (EC 3.2.1.41) (cat. M2, from *Bacillus licheniformis*, 400 U mL^{-1}) was obtained from Megazyme (Ireland). β-amylase (EC 3.2.1.2) (cat. 171580, from barley, 45 U mg^{-1}) was purchased from Merck (Belgium). 15-mL Conical centrifuge tubes (PP, cat. 430790) were obtained from Corning Incorporated (United States). 3.5-mL Green capped tubes (PP, cat. 1272777) were purchased from Greiner Bio-One (Germany). Cuvets (Hellma; 100-1-40 Cell 100-QS, 1 mm, 45 × 12.5 × 3.5 mm, 350 µL) were from Elcolab (The Netherlands). Micron Centrifugal Filter Devices with Ultracel YM-10 membrane were purchased from Millipore Corporation (United Kingdom).

5.2.2 Synthesis of 1-oxiranyloxy-2-hydroxypropyl starch (epoxy-starch)

AHP-WMS (10.00 g, 10% w/w H_2O, 0.051 mmol AGU, DS_{allyl} = 0.23) was suspended in a aqueous solution of Na_2CO_3 (1.75 mg, 0.017 mmol) and 350 mg $NaHCO_3$ (350 mg, 4.17 mmol), and CH_3CN (5 mL, 0.11 mmol). The suspension was stirred, while heating to 30°C. The reaction was initiated by adding H_2O_2 (35% wt., 9.4 mL 0.11 mmol) in portions of 100 µL during 30 min. After that, the mixture was stirred at 30°C for 6 h, then cooled down to room temperature, and finally water (25 mL) was added. The product was obtained by filtration on a glass filter (G3), and then subsequently washed with water (5 × 75 mL), ethanol (3 × 75 mL) and acetone (3 × 75 mL). The white powder, epoxy-WMS (9.52 g, 9.5% w/w H_2O, yield: 96%), was dried overnight at room temperature and stored at – 20°C.

5.2.3 Coupling of epoxy-starch with 1,6-hexanediamine

Epoxy-WMS (100 mg, 1 equiv.) was suspended in water (0.9 mL), followed by adding 5 equivalents of 66 mM 1,6-hexanediamine (HD, 7.67 g mL^{-1}) in water. The suspension was stirred for one night at 30°C. After cooling down to room temperature, the product was isolated using an Ultracel YM-10 membrane by centrifugation at 13,400 rpm for 1 h and washing with water (5 × 1 mL). After freeze-drying, a white powder was obtained (HD-WMS; 98 mg, yield: 98%).

5.2.4 Enzymatic degradation

For the enzyme preparation, α-amylase, pullulanase, amyloglucosidase and β-amylase were dissolved in deionized water purified by Millipore Milli-Q Gradient A10 (Millipore, United Kingdom), resulting in stock solutions containing 0.44, 0.40, 0.14 and 0.45 U μL^{-1}, respectively. The enzymatic digestion procedure for the degradation of native starch and its derivatives was as follows.

Starch (5 mg, 0.3 mmol) was dissolved in 1 mL sodium acetate buffer (0.05 M pH 5.0) and incubated with a combination of pullulanase (5 µL), α-amylase (5 µL) and amyloglucosidase (5 µL) at 40 °C for 18 h. Afterwards the solution was boiled for 10 min. For the β-amylase hydrolysis, starch (5 mg, 0.3 mmol) was dissolved in sodium acetate buffer (1 mL, 0.05 M pH 5.0), and incubated with β-amylase (5 µL) at 25°C for 18 h, and stopped by boiling for 10 min.

5.2.5 High-performance size-exclusion chromatography

High-Performance Size-Exclusion Chromatography (HPSEC) was carried out using a HPLC (ThermoFinnigan, United States), with three TSK-gel columns in series (7.5-mm × 300 mm per column, G4000PWXL, G3000 PWXL and G2500PWXL; TosoHaas, Japan), in combination with a PWXL-guard column (40 × 6 mm; TosoH, Japan). Elution took place at 30°C with 0.2 M $NaNO_3$ at a flow rate of 0.8 mL min^{-1}. After injection of a 100-µL sample, the eluens was analyzed with a refractive index detector (Shodex RI-101, Showa Denko K.K., Japan). The data were processed using Chromeleon software (Dionex, United States).

5.2.6 High performance anion exchange chromatography

High Performance Anion Exchange Chromatography (HPAEC) was performed on a HPLC system (Dionex, United States). The system was equipped with a quaternary gradient pump, an AS3000 autosampler complete with a helium degassing unit and an ED40 EC detector in pulsed amperometric detection (PAD) mode. The CarboPac PA1 column (2 × 250 mm; Dionex, United States) with a CarboPac PA1 guard column (2 × 50 mm; Dionex) was operated at a flow rate of 0.3 mL min^{-1} at 20°C. The gradient was obtained by solutions of mixing NaOAc (1 M in 0.1 M NaOH) with 0.1 M NaOH. 20-µL of ten-fold diluted sample was injected and a linear gradient to 0.5 M NaOAc in 0.1 M NaOH within 40 min was applied, followed by a linear gradient in 5 min to 1 M NaOAc in 0.1 M NaOH. Finally, the column was washed sequentially for 5 min with 1 M NaOAc in 0.1 M NaOH, and 20 min with 0.1 M NaOH. The data were processed using Chromeleon software (Dionex, United States).

5.2.7 MALDI-TOF Mass Spectrometry

Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) was carried out using an Ultraflex workstation (Bruker Daltonics GmbH, Germany) equipped with a 337 nm nitrogen laser. The mass spectrometer operated in the positive mode and was calibrated with a mixture of maltodextrins (mass range = 400-3500 Da). After a delayed extraction of 120 ns, the ions were accelerated with a kinetic energy of 25 kV. Hereafter, the ions were detected in the reflector mode. The lowest laser power required to obtain good spectra was used. A mixture of 1-µL ten-fold diluted sample and 1 µL of the matrix was dried on a sample plate. The matrix solution was prepared by dissolving 10 mg of 2,5-dihydroxybenzoic acid in a 1-mL mixture of acetonitrile:water (300 µL:700 µL).

5.2.8 Determination of epoxy groups

The amount of epoxy groups was measured using an adapted spectrophotometric assay as described previously.[14,15] All assays were performed in tubes (3.5 mL or 15 mL). Absorptions at 600 nm were measured using 1-mm cuvets in a Cary 100 UV-VIS spectrophotometer thermostated at 20°C, and followed with Cary WinUV Kinetic Software. epoxy-WMS, AHP-WMS as a control, and a calibration curve of 20 to 100 mM

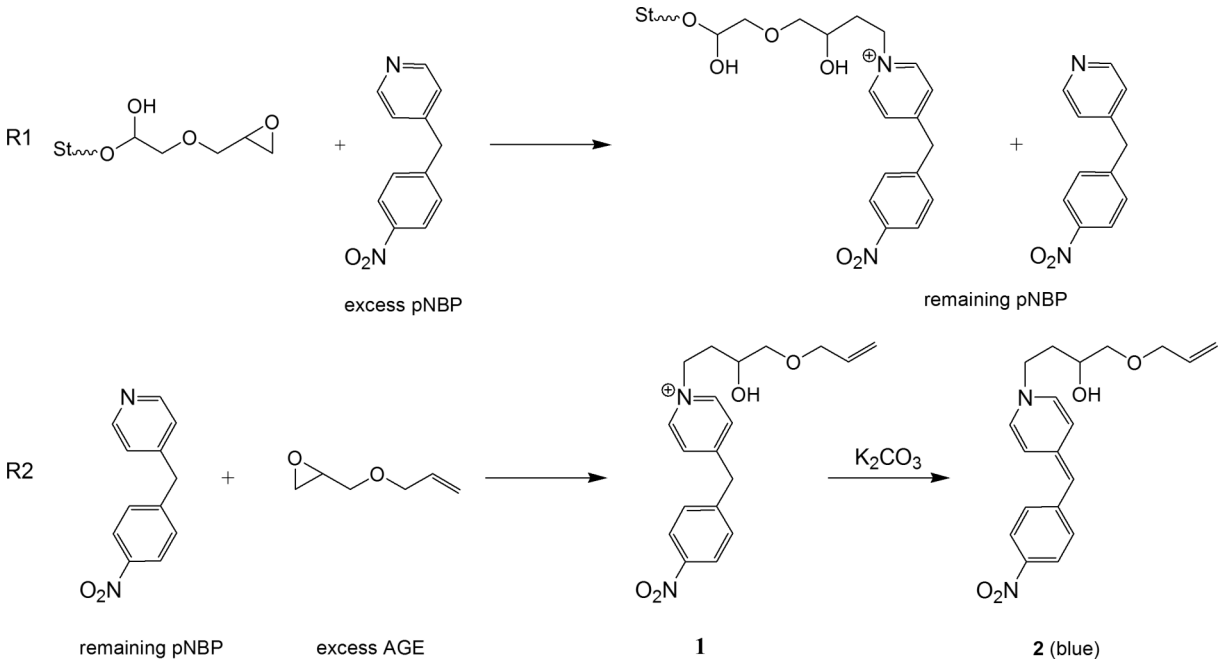
4-(4-nitrobenzyl pyridine) (*p*NBP) were used in the two step spectrophotometric assay. All assays were performed in triplicate, after which the epoxy content was calculated using the calibration curve and equation (5.1).

$$n_{\text{epoxy}} = n_{p\text{NBP}} - n_{\mathbf{2}} \tag{5.1}$$

where n_{epoxy}, $n_{p\text{NBP}}$ and $n_{\mathbf{2}}$ are the moles of epoxy groups on the modified starch, the number of moles of *p*NBP initially (Scheme 5.1, R1) added and the number of moles of the blue chromophore **2** of *p*NBP product **1** (Scheme 5.1, R2), respectively.

The two step spectrophotometric assay was performed as follows (Scheme 5.1).

Reaction 1 (R1): In a 15-mL tube, epoxy-WMS (0.1 - 0.3 g, 9.5% H_2O, 0.35 – 0.70 mmol AGU) was suspended in 2 mL of 90 mM Na_2HPO_4/NaH_2PO_4 buffer pH 7, containing 10% v/v DMSO. Freshly made 2 mL of 50 mM *p*NBP (100 µmol) in an ethylene glycol/ethanol mixture (80/20 v/v) was added to the suspension. After vortexing, the tube was placed in a heating bath at 80°C for 2.5 h. The reaction was cooled on ice for 10 min and centrifuged for 15 min at 15,200 rpm.



Scheme 5.1. Two step spectrophotometric assay to quantitative epoxy groups in modified starch; *p*-nitrobenzyl pyridine (*p*NBP) product (**1**) and blue chromophore (**2**).

Reaction 2 (R2): The supernatant was analyzed for the amount of remaining (unreacted) *p*NBP as follows. 1 mL of the supernatant was pipetted in a 3.5-mL tube and mixed with 1-mL freshly made 50 mM AGE (50 µmol) in 90 mM Na_2HPO_4/NaH_2PO_4 buffer pH 7, containing 10% v/v DMSO. Again, the reaction was performed in a heating bath at 80°C for 2.5 h. The samples were cooled on ice for 10 min and diluted fourfold. Before blue color development with 100 µL 1 M K_2CO_3, the diluted mixture (200 µL) was mixed with 200 µL ethanol. The sample was rapidly mixed with K_2CO_3 and the absorption at 600 nm (A_{600}) was measured after 1 min.

5.2.9 Amino substitution

The nitrogen (N%) and carbon contents (C%) of HD-WMS and epoxy-WMS were obtained using a Fisons EA1108 elemental analyzer. In the DS_{HD} calculation, we assumed that only one amino group of hexanediamine (HD) binds to an epoxy group, leaving one amino group free. DS_{HD}, expressed as moles amino groups per mole glucose units, was determined using *n*N (N%/MW; N-atom MW = 14,01), *n*C (C%/MW; C-atom MW = 12,01), and the number of atoms in the sample according to equation (5.2):

$$\frac{n\mathrm{C}_{\mathrm{HD}}}{n\mathrm{N}_{\mathrm{HD}}} = \frac{\mathrm{C}_{\mathrm{AGU}} + \mathrm{C}_{\mathrm{AGE}} \cdot \mathrm{DS}_{\mathrm{allyl}} + \mathrm{C}_{\mathrm{HD}} \cdot \mathrm{DS}_{\mathrm{HD}}}{\mathrm{N}_{\mathrm{HD}} \cdot \mathrm{DS}_{\mathrm{HD}}} \tag{5.2}$$

where $n\mathrm{C}_{\mathrm{HD}}$ is the moles of C-atoms in HD-WMS; $n\mathrm{N}_{\mathrm{HD}}$ is the moles of N-atoms in HD-WMS (*n*N minus the *n*N from N-containing compounds of epoxy-WMS proportionately); $\mathrm{C}_{\mathrm{AGU}}$, $\mathrm{C}_{\mathrm{AGE}}$ and C_{HD}-atoms correspond to the number of C-atoms in AGU (in starch), in AGE or HD, respectively; N_{HD} is the number of N-atoms in hexanediamine. $\mathrm{DS}_{\mathrm{allyl}}$ of AHP-WMS is 0.23.

5.2.10 Physicochemical properties

The temperature of gelatinization of the modified starches was measured using a Differential Scanning Calorimeter (DSC) (Perkin Elmer DSC-7, Boston, USA). Sample preparation and analysis were performed as described previously.[13] The PE Pyris – DSC-7 software was used for data handling. Onset temperature (T_o), peak temperature (T_p), completion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were calculated from

the DSC thermogram. ΔH_{gel} was based on the dry material (J g^{-1} dry starch). The swelling power (SP) and solubility index (SI) of starches was determined using the previously published method.[13] The experiment was repeated three times. The mean values are reported, with standard deviation.

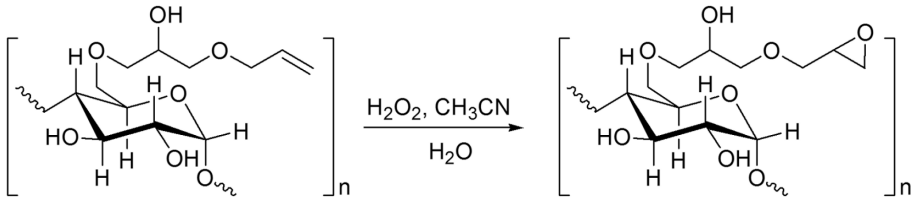
5.3 Results and discussion

5.3.1 Synthesis of epoxy starch derivatives

The double bonds of granular AHP-WMS were epoxidized using ten equivalents of H_2O_2 and CH_3CN in slightly alkaline suspension at 30°C (Scheme 5.2). The combination of hydrogen peroxide and acetonitrile is a well-known reagent for the epoxidation of carbon double bonds.[16] A two step colorimetric assay was developed to determine the amount of epoxy groups in the product. Furthermore, epoxy-WMS was subjected to enzymatic digestion to characterize the structure of the starch derivative.

5.3.2 Quantitative analysis of epoxy groups

The amount of epoxy groups was determined using a quantitative spectrophotometric assay.[14] In this method, *p*-nitrobenzyl pyridine (*p*NBP) was used to assay epoxides through the formation of a blue chromophore. This assay was tested with epoxy-WMS and *p*NBP, but the colorimetric analysis of the starch suspension was impossible due to the insoluble nature of the starch. Therefore, a new two step spectrophotometric assay was developed (Scheme 5.1). In the first step, an excess of *p*NBP was used to quantitatively convert all epoxide groups on the starch (Scheme 5.1, R1). A dark blue/green starch derivative was obtained. Subsequently, a small part of the supernatant containing the remaining *p*NBP was transferred to a tube with a high concentration of allyl glycidyl ether (AGE). In this tube, the remaining *p*NBP was converted in the *p*NBP product **1** (Scheme



Scheme 5.2. The synthesis of epoxy starch derivatives.

Table 5.1. Analysis of epoxy-WMS and hexanediamine (HD) treated epoxy-WMS

Sample	Epoxy groups (mmol g^{-1})	DS	DS/DS_{allyl} (%) [b]	C (%) [c]	N (%) [c]
Epoxy-WMS	0.13 ± 0.03	0.025 [a]	11 [a]	43.08	0.24 [d]
HD-WMS	n.d. [e]	0.026 [c]	11	39.09	0.53

[a] Determined using *p*-nitrobenzyl pyridine titration; [b] DS_{allyl} = 0.23; [c] Determined using elemental analysis; [d] Derived from proteins which are present in WMS. [e] Not determined.

5.1, R2). The *p*NBP product is deprotonated in a basic medium using K_2CO_3, giving the blue chromophore **2**. The concentration of chromophore **2** was determined by its absorption at 600 nm. The amount of *p*NBP initially added (R1) minus the amount of **2** formed (R2) equals the amount of epoxy groups on the starch. With this assay, DS_{epoxy} of 0.025 was determined corresponding to 0.13 ± 0.03 mmol epoxy groups per g dry starch (ds). (Table 5.1). This suggests that 11% of the allyl groups were converted into epoxy groups. The controls showed hardly any differences in A_{600}.

5.3.3 Amine substitution

Epoxy groups on a surface are able to react with nucleophiles such as NH_2- and OH-groups. Using hexanediamine (HD), the binding of NH_2-groups to epoxy groups was investigated. In this reaction, we assume that one amino group of HD reacts with an epoxy group since there is a ten-fold excess of amino groups. The amount of NH_2-groups in HD-WMS was determined using an elemental analyzer (Table 5.1). Via equation (5.2), a DS_{HD} of 0.026 was obtained. This result corresponds to DS_{epoxy} obtained by the colorimetric assay, which means that every epoxy group in epoxy-WMS has reacted with HD.

5.3.4 Physicochemical properties

The structural changes upon epoxidation of AHP-starch may affect the physicochemical properties such as gelatinization, swelling and solubility of the granules. Hardly any differences in the gelatinization temperatures between AHP-WMS and epoxy-WMS were observed (Table 5.2). However, both the swelling power and solubility index decreased after epoxidation of the allyl groups. This suggests that cross-links may have been formed in the starch, although no significant increase of gelatinization temperatures was obtained. In previous research, cross-linked starches were shown to exhibit reduced swelling and solubility of the granules, but also increased gelatinization temperatures.[17,18]

Table 5.2. Thermal properties,[a] swelling power and solubility index of native starch and its derivatives

Sample	T_o [a]	T_p [a]	T_c [a]	ΔH_{gel} [a]	Swelling power (g g^{-1})	Solubility index (%)
WMS	57.3	69.4	75.7	13.8	4.5 ± 0.2	0.2 ± 0.0
AHP-WMS	49.1	56.6	65.5	5.5	14.7 ± 1.0	7.8 ± 1.0
Epoxy-WMS	50.3	56.5	64.7	4.9	12.9 ± 0.2	5.4 ± 0.2

[a] Measured by Differential Scanning Calorimetry; T_o, T_p and T_c are the onset, peak and completion temperature respectively, and ΔH_{gel} is the enthalpy of gelatinization.

5.3.5 Structural characterization of epoxy-WMS

The results of the spectrophotometric assay of epoxy groups, and the elemental analysis of the HD reaction, imply that only a small amount of epoxy groups is available for binding to nucleophilic groups. Furthermore, reduced swelling and solubility of epoxy-WMS suggest that subsequent reactions may have taken place, such as the formation of internal cross-links in the starch granule. To study the structure of epoxy-WMS, the modified starch was enzymatically hydrolyzed. The enzymatically degraded products will give information about the differences in structure between AHP-WMS and epoxy-WMS.

The extent of β-amylase hydrolysis of native starches and its derivatives was studied from HPAEC and HPSEC elution profiles. These profiles showed that the hydrolysis of the AHP-WMS and epoxy-WMS liberated less maltose than the native starch (results not shown). Furthermore, the relative amount of liberated maltose was determined at 89% for AHP-WMS and at 73% epoxy-WMS. This suggests that enzymatic degradation of epoxy-WMS was more sterically hindered due to the presence of intra- or intermolecular ether cross-links. According to other studies, cross-linked starch derivatives are less accessible for enzymatic hydrolysis than their etherified starches.[17,18]

5.3.6 Enzymatic digestion with pullulanase, α-amylase and amyloglucosidase

To study the structure of epoxidized WMS in more detail, epoxy-WMS, AHP-WMS and non-modified starch were also subjected to simultaneous enzymatic digestion using pullulanase, α-amylase and amyloglucosidase as described before.[12] The HPSEC elution profiles of both WMS derivatives showed high molecular weight fragments compared to the native starch (results not shown), i.e. the enzymes were sterically hindered by both

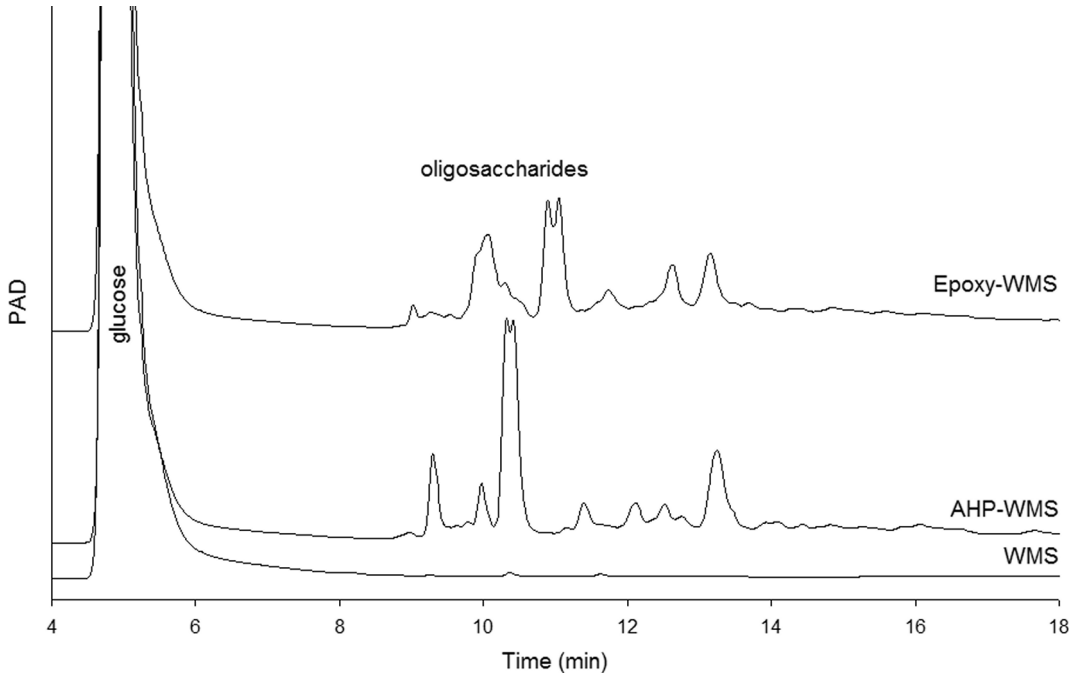


Figure 5.1. HPAEC elution profiles of oligomers mixture of WMS, AHP-WMS and epoxy-WMS obtained after enzymatic degradation by pullulanase, α-amylase and amyloglucosidase.

WMS derivatives. A different oligomeric distribution was obtained for epoxy-WMS than for AHP-WMS.

Likewise, both HPAEC elution profiles of AHP-WMS and epoxy-WMS (Figure 5.1) show high molecular weight oligomers which are resistant to further enzymatic digestion. All fragments were eluted within 20 min. Again, a different molecular weight distribution of the enzymatically generated oligomeric fragments was obtained for AHP-WMS and epoxy-WMS. The oligomeric fragments of AHP-WMS eluting at 9.3 min, between 10.3-10.4 min and at 12.2 min disappeared from the profile of digested epoxy-WMS, whereas other enzyme-mediated degradation products appeared at 10.1 min, 10.8-11.4 min and 12.7 min.

The different mass distribution of the oligomeric fragments of epoxy-WMS and AHP-WMS was determined in more detail by MALDI-TOF MS (Figure 5.2) In the MALDI-TOF MS spectrum of AHP-WMS, enzyme-resistant oligosaccharides with various degrees of polymerization (DP) were identified, ranging from maltose (DP2) to DP9 with one to five allyl groups (AHP). The MS spectrum of epoxy-WMS showed a regular pattern with more and diverse oligomers (DP ranging from two to seven). The mass distribution of

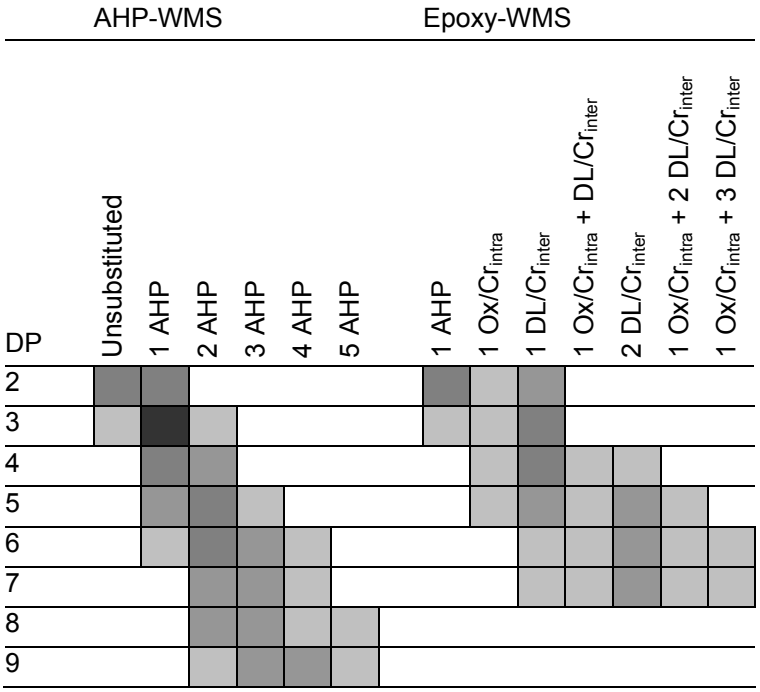


Figure 5.2. Mass distribution of the oligomeric fragments of AHP-WMS and epoxy-WMS. Enzyme-resistant oligomeric fragments of AHP-WMS contain allyl groups (AHP), whereas fragments of epoxy-WMS contain AHP or epoxy groups (Ox) as well as diol groups (DL) and cross-links within the oligomer (Cr_{intra}) or between two fragments (Cr_{inter}). DP: degree of polymerization. The total signal intensities of the oligomers in a certain DP are normalized to 100%. Relative intensities 0 100%

epoxy-WMS included several different enzyme-resistant oligomers having epoxy groups (Ox) and unreacted AHP groups. In addition, oligomeric fragments containing diol groups (DL) were found. Furthermore, there were fragments possessing internal cross-links (Cr_{intra}) or between two different fragments (Cr_{inter}). These oligomers with diols and cross-links were a result of subsequent reactions of epoxy groups, such as hydrolysis or the formation of intra- and intermolecular bridges with free hydroxyl groups in starch.

Some possible different enzyme-resistant oligomeric DP3 fragments obtained after enzymatic hydrolysis of epoxy-WMS are illustrated in Figure 5.3. Fragments containing epoxy groups (Ox) and cross-links within the oligomer (Cr_{intra}) have the same mass over charge ratios, as is illustrated in Figure 5.3. Thus, they are indistinguishable in the MS analysis. Similarly, the oligomers having diols (DL) and cross-links between two fragments (Cr_{inter}) give the same mass over charge ratios in the MS spectrum of epoxy-WMS. The diversity of possible enzymatically degraded fragments becomes larger for

oligomers containing more than three glucose units. The fragments at higher mass over charge ratios (Figure 5.2) could not be unambiguously assigned due to their large diversity, low relative intensities and overlapping of signals.

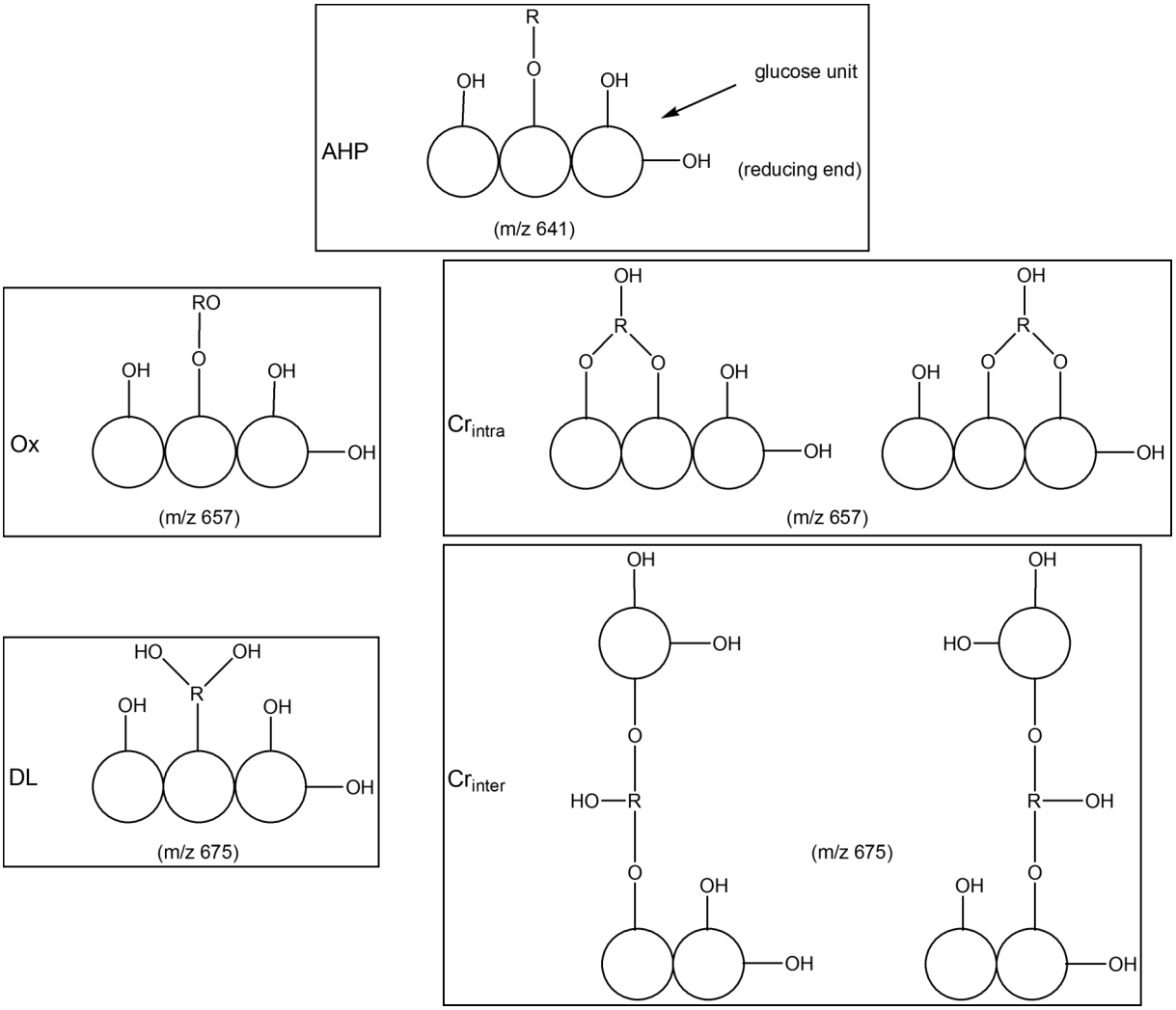


Figure 5.3. Simplified scheme of some possible different enzyme resistant DP3 fragments of the pullulanase, α-amylase and amyloglucosidase hydrolysates of epoxy-WMS. Substituted DP3 fragments may contain one allyl group (AHP, *m/z* 641), an epoxy group (Ox, *m/z* 657), a diol group (DL, *m/z* 675), a cross-link within the oligomer (Cr_{intra}, *m/z* 657) or a cross-link between two fragments (Cr_{inter}, *m/z* 675). For reasons of clarity, only fragments with a substituent at the central glucose unit are drawn. DP: degree of polymerization; R: AHP group; RO: epoxidized AHP group.

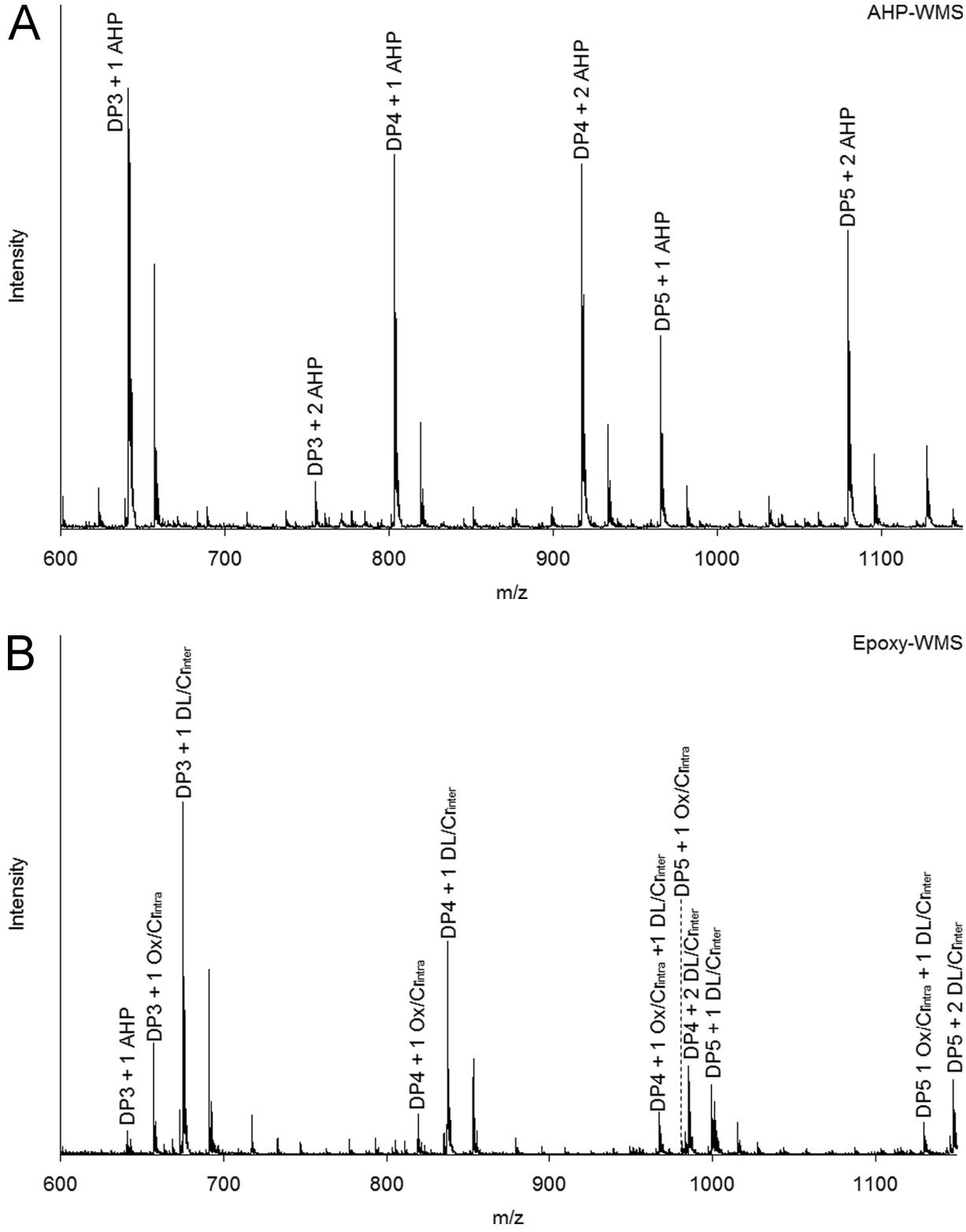


Figure 5.4. Enlargement of MALDI-TOF mass spectra of the pullulanase, α-amylase and amyloglucosidase hydrolysates of AHP-WMS (A) and epoxy-WMS (B). Enzyme-resistant oligomeric fragments of AHP-WMS contain allyl groups (AHP), whereas fragments of epoxy-WMS contain AHP or epoxy groups (Ox) as well as cross-links within the oligomer (Cr_{intra}) and between two fragments (Cr_{inter}), or diol groups (DL). DP: degree of polymerization.

In the enlargements of the MALDI-TOF MS spectra (Figure 5.4), differences between the oligomers obtained after enzymatic degradation of AHP-WMS and epoxy-WMS, respectively, are clearly shown. The fragment (DP3) containing one AHP group is still present after epoxidation. Next to this oligomer, its epoxidized DP3 or DP3 with Cr_{intra} is identified at the signal of 657 *m/z*, followed by DP3 with Cr_{inter} or DL (*m/z* 675). These differences in oligomeric fragments are also obtained for fragments containing four or five glucose units. Moreover, DP4 and DP5 with two Cr_{inter} or DL, and with one epoxy group or Cr_{intra}, and one cross-link between two fragments or a diol group are found for epoxy-WMS. Furthermore, DP5 with one Ox group or Cr_{intra} and two Cr_{inter} or DL was identified. in the MS analysis.

These results show significant differences in the oligomeric fragment patterns of AHP-WMS and epoxy-WMS. Small to large fragments with several allyl groups were obtained for AHP-WMS, whereas a regular pattern of more and diverse oligomers was found for epoxy-WMS. A small number of oligomers carrying unmodified AHP groups and epoxy groups were found in the MALDI-TOF MS of epoxy-WMS. Most fragments were identified as fragments decorated with diol groups and/or with ether cross-links generated, respectively, by hydrolysis of the epoxy moieties or by the formation of covalent bonds within the oligomer or between two different fragments. These subsequent fragments may have been formed during the epoxidation or storage of the compound, but it might also be possible that these fragments were generated during the enzymatic digestion. This suggests that a larger amount of allyl groups in AHP-WMS was converted into epoxy groups than determined with *p*NBP test, but a significant amount of the epoxy groups reacted further with nucleophilic OH-groups in glucose units or water to generate cross-links or diols, respectively.

5.4 Conclusions

In the synthesis of epoxy starch derivatives, allyl groups were converted into epoxy groups using hydrogen peroxide and acetonitrile. A two step spectrophotometric assay was developed to obtain the amount of epoxy groups in starch. Subsequently, a DS_{epoxy} of 0.025 was determined corresponding to a quantity of epoxy groups of 0.13 mmol g^{-1} ds. Epoxy starch derivatives exhibited slightly reduced enzymatic susceptibility, swelling capacity and solubility compared with AHP-WMS. Enzymatic degradation elucidated that a larger amount of allyl groups of AHP-WMS was converted into epoxy groups than

determined in the colorimetric assay. However, only a small amount of epoxidized fragments were found, due to secondary reactions of the reactive epoxy groups, like intra- and intermolecular cross-linking with free hydroxyl groups of polysaccharide chains, and hydrolysis.

Acknowledgements

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Chapter 6

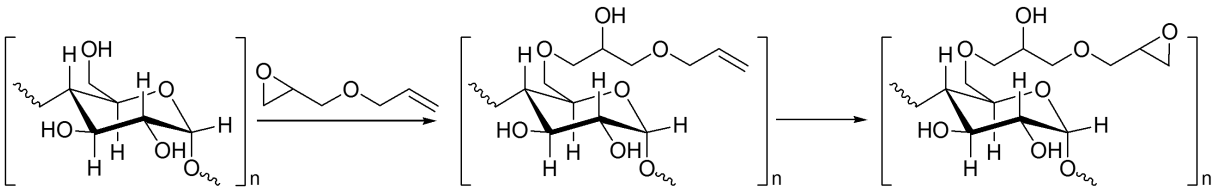
Summary and general discussion

6.1 Introduction

Starch is widely used as raw material in numerous commercial applications, e.g. in the paper, textile, food and pharmaceutical industries. Several characteristics, such as biodegradability, ease of modification, and low costs make this polymer an excellent material for industrial use. In order to increase its industrial use and to fulfill the various demands for functionality, starch is often modified by a number of chemical or physical methods. The introduction of a functional group into starch leads to marked changes in the properties and behavior of this biopolymer, depending on the degree of substitution (DS) and the type of functional group.[1] Many commercial starch derivatives are chemically manufactured to improve specific properties, such as increased paste consistency, cold storage stability, decreased gelatinization and adjusted gelling properties.[2,3] The modified starches generally have a DS of around 0.2. They comprise a wide range of industrial products, such as drug delivery systems in pharmaceutics, stabilizers and thickener agents in food products, and sizing agents in paper and textile (Chapter 1).

An area of growing interest is the development of granular multifunctional starch derivatives for applications as biodegradable delivery system. These starch derivatives require a functional group which has the ability to bind to a wide range of ligands. Amongst these starch-based products are derivatives containing epoxy groups since they are highly reactive towards compounds having amino and hydroxyl groups such as peptides, amino acids and polyols. Moreover, epoxy groups could form cross-links within starch granules to generate matrices, which can be used for physical entrapment of agrochemicals such as insecticides or bioactive compounds such as proteins. The functionalization of starch with epoxy groups can be accomplished by different strategies. Direct integration of epoxy groups can be achieved using epichlorohydrin and bisepoxides.[4-6] Alternatively, epoxy starch derivatives can be synthesized in a two step process, via etherification of the starch with allyl reagents and subsequent epoxidation of the double bonds.[7-9]

The objective of the research described in this thesis was to develop and optimize a mild and environmentally friendly process for the synthesis of novel highly reactive granular epoxy starch derivatives via the two step method (Scheme 6.1). An experimental design approach was utilized to optimize process conditions of the first step. Furthermore, the



Scheme 6.1. Synthesis of epoxy starch derivatives.

properties and behavior of starch were studied in detail to gain knowledge about the chemical structure of the modified starch and its physicochemical properties. Subsequently, the allyl groups in the starch derivatives were epoxidized.

6.2 Research: summary and discussion

Synthesis of allyl starch derivatives

Conventional syntheses of allyl starch derivatives utilize allyl halogenides in the presence of catalysts at temperatures above the gelatinization temperatures of starch.[10,11] As allyl halogenides are insoluble in aqueous media, the syntheses are usually performed in polar aprotic media. These conditions have drawbacks like formation of large quantity of salts and difficulties in product isolation due to the gelatinization process.

Alternatively, the allyl groups can be introduced into starch by the reaction with epoxyalkyl alkenyl ethers under mild conditions (Chapter 2).The reaction of allyl glycidyl ether (AGE) and granular waxy maize starch (WMS) or amylose-enriched maize starch (AEMS) was studied (Scheme 6.1). This modification was performed in slightly alkaline suspension at temperatures below the gelatinization temperatures of maize starches. Sodium sulfate was present to prevent gelatinization of the granule and to maintain the granular form of the starch. After reaction, the product, 1-allyloxy-2-hydroxylpropyl-starch (AHP-starch), was simply isolated via filtration. Via ^{1}H NMR analysis, DSs of 0.19 and 0.20 were determined for AHP-WMS and AHP-AEMS, respectively.[12] This indicates a very good availability of AGE inside the granule for WMS and AEMS under these heterogeneous reaction conditions.

Moreover, it was shown that factors such as amylose to amylopectin ratio, intragranular packing and the presence of lipids mainly govern the DS.[3] Since reagents with different reactivity penetrate into the granule with different efficiencies, the organization of the granule influences the extent and the distribution of the substitution.[13] When starch is modified with highly reactive reagents such as acetic anhydride, higher DS

values are found for small size granule fractions than for larger starch granules. In the reaction of starch with a slow reacting reagent such as propylene oxide, the granule size is not of importance.[3,13,14] Generally, chemical derivatization starts in the more amorphous regions of the granule (composed of mainly amylose and the branching point of amylopectin) and proceeds to the more crystalline regions.[15-17] Amylose tends to become more substituted than amylopectin.[13] In general, the results of the chemical modifications depend on the granule structure, reaction conditions and starch source.[16]

Additionally, the characterization of AHP-starch was performed using 1D and 2D NMR. This analysis suggested that some of the glucose moieties in starch were substituted at the O-6 position. This information was validated with AHP substituted methyl-α-glucopyranoside.[12] Generally, etherification of starch with epoxides favors O-2 monosubstitution above O-3 and O-6 substitution in the presence of a low amount of base.[18] Although the 2D NMR analysis indicated an O-6 substitution, other techniques such high performance liquid chromatography (HPLC) and capillary gas chromatography-mass spectroscopy (GC-MS) should give further the information about the location of the substituent on the glucose units.

Information about the distribution of AHP groups along the polysaccharide chains was obtained using enzymatic degradation studies. AHP-AEMS (containing 70% amylose) and AHP-WMS (mainly amylopectin) were subjected to enzymatic degradation by pullulanase, α-amylase and amyloglucosidase followed by analysis of the hydrolysis products using high performance anion exchange chromatography (HPAEC) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Oligomers resistant to further enzymatic hydrolysis indicated that AHP groups on the modified starches constitute sterical hindrance to α-amylase and amyloglucosidase and the enzymatic hydrolysis was stopped close to these modified glucose units. The size and the substitution pattern of the enzyme-resistant oligomers showed significant differences between the AHP substituted starches (Chapter 2). Modified amylose-rich starch showed a more heterogeneous distribution of AHP groups along the polysaccharides chains and larger fragments with clustered groups, whereas modified amylopectin-rich starch was more uniformly substituted and displayed a homogeneous distribution of the AHP groups over the polymer. Considering these differences of substituent distribution of AHP-AEMS and AHP-WMS, it is concluded that the substitutions has taken place preferentially in the

amorphous regions of the starch granule.[2,18] Other substitution distribution studies have confirmed that the amorphous regions are more accessible to modifying reagents than the crystalline regions.[2,3,13,19]

Optimization study

The optimization of the process conditions for the synthesis of AHP-WMS and AHP-MS was studied using a statistical experimental design approach (Chapter 3). An experimental design approach is used for scientific and engineering experiments to improve the production process, leading to improved process yields, reduced overall costs or time. In the study towards the optimization of the chemical process, the influence of the process conditions on the DS of AHP-starch was investigated. In the screening phase, the most important process factors were identified followed by the optimization of the synthesis based on the significant factors found in the screening.

Three different starches were modified during this optimization study, namely WMS, MS and AEMS containing 0.9%, 27% and 70% amylose respectively. In the screening phase, six variables at three levels were selected (Table 6.1), leading to 35 experimental conditions. Subsequently, the experiments were performed and DS was determined. However, in many cases for AEMS it was not possible to determine the DS due to a too high viscosity of the modified starch. As a consequence, no reliable statistical analysis

Table 6.1. Experimental design for the synthesis of AHP-WMS and AHP-MS: Six variables at three levels [a], and the optimal conditions for the best conversion of WMS and MS with a maximum degree of substitution (DS), and with a maximum DS at the lowest amount of allyl glycidyl ether (AGE).

	min-max levels			WMS		MS	
Variables	-1	0	1	Max. DS [e]	Lowest AGE [f]	Max. DS [e]	Lowest AGE [f]
C_{starch} (g kg^{-1} slurry) [b]	200	300	400	300	300	300	300
Reaction time (h)	4	10	16	4.0	4.0	16.0	16.0
Temperature (°C)	20	34	48	34.2	34.2	37.0	37.0
NaOH (% ds) [c]	0.4	0.7	1.0	0.99	0.99	0.99	0.99
Na_2SO_4 (% ds) [c]	5	20	35	20	20	20	20
AGE (% ds) [c]	0.0016	0.0091	0.0166	0.0166	0.0016	0.0099	0.0031
DS	-	-	-	0.102	0.096	0.039	0.031
Conversion eff. (%) [d]	-	-	-	4.3	42.1	2.8	7.1

[a] The levels are indicated with 1, 0 and -1. [b] Starch concentration. [c] Variables are calculated as % (w/w) on dry starch (ds). [d] Efficiency. [e] No constraints of the process factors. [f] Max.DS and no constraints for the other factors.

could be performed and AEMS data had to be left out for further research. The statistical reports for WMS and MS showed that the temperature had a pronounced effect for both starches. The effect of the starch concentration (C_{starch}) and the amount of Na_2SO_4 was negligible. For the final optimization model the reproducibility of the model was investigated in more detail.

In the improving phase, the variables of starch concentration and the amount of Na_2SO_4 were fixed at 300 g kg^{-1} and 20% ds, respectively. The other four variables were evaluated in the Central Composite experimental Design (CCD). Twenty-five additional experiments were generated, performed and analyzed. The obtained DS were significantly lower than the DS values in the screening phase, due to different starch batches. This batch effect was normalized in the statistical analysis. The analysis showed that the temperature had the largest effect on the modification of both starches. The NaOH and AGE concentration were of influence on the DS, but less pronounced.[20]

Based on these results, the optimum conditions for the highest DS or highest DS at low cost for WMS and MS were generated using the specific module of the statistical program (Table 6.1). The optimal generated conditions for the synthesis of AHP-WMS and AHP-MS differ extremely in the maximum DS and the conversion efficiency. These differences can be explained by differences in the amount of AGE needed for the synthesis and the accessibility of starch for AGE. Furthermore, amylopectin-rich starch was substituted to a higher extent than starch with lower amylopectin content. This is logical since the rate and efficiency of chemical modification depends on the reagent type, botanical source of native starch and the size and structure of the granule.[3,19,21]

The experiments with the highest DS in the screening were repeated on large scale for WMS, MS and AEMS (1 or 3 kg, Table 6.2). The results show that the extent of starch modification with AGE depends on the type of maize starch. Amylopectin-rich starch was

Table 6.2. Experiments conditions for a large batch of WMS, MS and AEMS.

Starch	Amylose (%)	Weight (kg)	C_{starch} [a]	t (h)	T (°C)	NaOH [b]	Na_2SO_4 [b]	AGE [b]	DS
WMS	0.9	3	400	4	48	1	35	0.0016	0.23
MS	27	1	400	16	48	1	35	0.0166	0.11
AEMS	70	1	400	16	48	1	35	0.0166	0.13

Variables are calculated as [a] g per kg slurry or [b] % (w/w) on dry starch

substituted to a high extent in a short time compared to starch with lower amylopectin content (and therefore higher amylose content).

Physicochemical properties

The physicochemical properties of the modified starches were initially investigated using the large batches and the samples of the screening phase (Chapter 3). The properties and behavior of starches were affected by the incorporation of AGE onto the starch granule (Chapter 4). After etherification, the swelling and solubility of the starch granule increased. Microscopic measurements confirmed the swelling capacity of the three starches in order of AHP-WMS > AHP-MS > AHP-AEMS.[22] Moreover, the swelling power and solubility index of AHP-WMS and AHP-MS increased with an increasing DS until a saturation limit. Presumably, only a small difference in swelling capacity for modified starches was induced at higher temperature, due to the already high swelling capacity at room temperature as reported previously for other etherified starches.[3,23] Correlated to the swelling of the starch granule, the modified starches had decreased gelatinization temperatures in comparison to the native starch. These differences were ascribed to the influence of AHP groups on the interactions between the starch chains. These structural changes in the starch granule also induced differences in pasting behavior. The altered pasting properties led to shear thinning during heating and a better gel network after cooling for AHP-WMS and AHP-MS. Again, modification method, reaction conditions and starch sources are factors that govern the pasting behavior of starch paste.

Granules with an increased swelling power can induce a desirable change in the flow behavior under shear, making them interesting for biomaterials. A qualitative difference in the behavior of modified and native starch suspensions under shear was demonstrated by using a counter-rotating rheo-optical setup (Chapter 4).[24] Immersed into an immiscible Newtonian fluid, droplets of AHP modified WMS and MS suspension (100% volume fraction, maximal swelling) broke up into several smaller droplets under shear, whereas the native starches did not elongate but only form a “tail” or only rotated in the vorticity direction.[22] The different behavior of the droplets of modified and native starch is related to different swelling power of the granules and thus polymer concentration and granule rigidity (governing the suspension viscosity). The behavior of the modified starch is quite

similar to the one of a viscous liquid droplet immersed into an immiscible matrix. The absence of the break up of the droplet of native starch suspension may be explained by the liquid between the granules which can block the deformation and break up due to interfacial tension between the water and the matrix fluid.[25]

Synthesis of epoxy starch derivatives

Synthesis of epoxy starch derivatives starting from the AHP-starch was subsequently studied. Epoxidation of double bonds in allyl carbohydrates is generally achieved using hydrogen peroxide or peracids. A high degree of epoxidation of allyl cellulose by peracid was reported, although a low molecular weight of the derivative was obtained.[8] In our work, a successful introduction of epoxy groups was accomplished by epoxidation of double bonds in granular AHP-WMS (Scheme 6.1) using hydrogen peroxide and acetonitrile (Chapter 5). For a quantitative determination of the formed epoxy groups, a two step spectroscopic method was developed. *p*-Nitrobenzyl pyridine (*p*NBP) was used to assay epoxy groups through the formation of a blue adduct. Using this colorimetric assay, DS_{epoxy} of 0.025 was obtained corresponding to 0.13 mmol epoxy groups per gram dry starch (ds). It was expected that more epoxy groups were formed since the DS of the starting material was 0.23. Presumably, the epoxidation yield was higher but the epoxy groups are not stable under the applied reaction conditions. Indeed, the reduced swelling power and the solubility index of Epoxy-WMS indicated that extra cross-links could have been formed upon reaction.

To elucidate these structural changes, the differences in structure between AHP-WMS and epoxy-WMS were studied by simultaneous enzymatic degradation using the hydrolytic enzymes as previously described (Chapter 2) followed by MS analysis. Epoxy-WMS had a different distribution of oligomers in the MALDI-TOF MS spectrum than AHP-WMS. Although small AHP fragments were detected in the substitution pattern of epoxy-WMS, more and diverse enzyme resistant oligomers were revealed than in the case of AHP-WMS. This suggests that AHP-WMS was initially highly epoxidized. However, a small amount of epoxidized fragments were found than we expected, due to secondary reactions of the reactive epoxy groups such as intra- and intermolecular cross-linking of polysaccharide chains and hydration.

6.3 Future perspectives and alternative approaches

In the course of the present study, several investigations were set up to synthesize and characterize epoxy starch derivatives. Via a two step method, maize starches were modified with allyl glycidyl ether followed by epoxidation of the double bond. Most of the studies were focused on the reaction of maize starches and allyl glycidyl ether to gain knowledge about the mechanism of the starch modification and to control the process conditions. In principle, a range of epoxyalkyl alkenyl ethers with variable chain length can also be used for the synthesis of allyl starch derivatives. The spacer length may influence hydrophobic-hydrophilic interactions, crystallinity and rheology of the modified starch as well as reactivity of the epoxy groups attached to the starch. It is expected that a longer spacer leads to a reduced cross-linking and therefore to a higher DS of epoxy groups in the final products. Therefore, it is of interest to study the synthesis and properties of (epoxy) starch derivatives with a longer spacer (Figure 6.1).

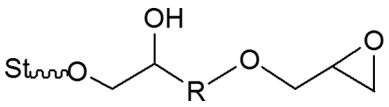


Figure 6.1 Epoxyalkyl alkenyl starch derivative; St: starch; R: alkyl group with or without one or two O-atoms or other groups such as amide groups.

In the study towards the characteristics of the modified starch, the extent and position of the introduced groups were studied by NMR . Such first insight information can be supplemented by hydrolysis and HPLC analysis. Additionally, GC-MS can be applied to determine the complete monomer composition of hydrolyzed and/or pretreated starch fragments. Moreover, this technique can provide additional structural information. At the polymer level, the substituent distribution of AGE in amylose-rich and amylopectin-rich maize starch were investigated. The observation was made that substitutions have taken place preferentially in the amorphous regions of the starch granule. Further investigation can be explored at granular level to gain detailed information about the accessibility of AGE for the different maize starch granules and its distribution over amylose or amylopectin.

Knowledge about these structural changes in the starch granule can be of importance for understanding the altered properties, and for the development of specific properties. The incorporation of a small amount of AGE led to altered physicochemical properties of starch. A saturation limit of the swelling capacity for AHP-starch with increasing DS was obtained. This tendency may be investigated through studying other properties such as temperature of gelatinization and viscosity of starch with a different DS.

Epoxy starch derivatives were synthesized by the conversion of double bonds in AHP-WMS into epoxy groups. Although successful, the epoxy starch derivatives had a low content of epoxy groups, due to subsequent side reactions. Therefore, more studies are needed to find optimal conditions that allow higher conversion and yield, and a high content of epoxy groups. A simpler route to synthesize epoxy starch derivatives might be the use of diepoxides. We have explored this option in a model system consisting of methyl-α-glucopyranoside (MG) as a model compound, and 1,3-butadiene diepoxide, 1,2,5,6-diepoxyhexane and 1,2,7,8-diepoxyoctane. The isolated products of each reaction appeared to be a very complex mixture of mainly ring-opened and cyclized diepoxides (Figure 6.2, for the case of 1,2,5,6-diepoxyhexane)[26] and unreacted MG, as shown by HPLC and LC-MS analysis. No epoxy methyl-α-glucopyranoside derivatives were identified. This indicated that diepoxides rather react with themselves than with another compounds under the conditions of the experiments. Therefore, substitution of starch with diepoxides is not an option for the synthesis of epoxy starch derivatives with high yield. These results show that the two step synthetic route explored in this thesis is the only viable route for the synthesis of epoxy derivatives of starch and other carbohydrates.

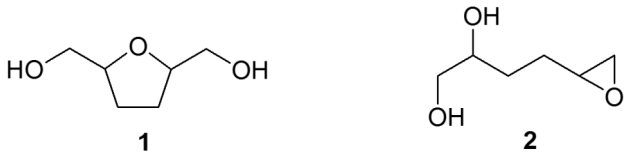
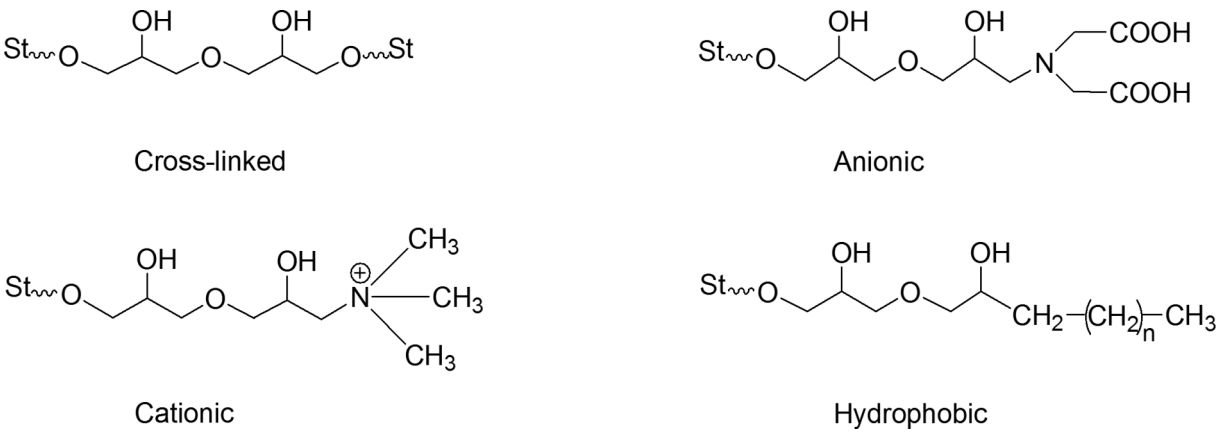


Figure 6.2. Main products of 1,2,5,6-diepoxyhexane after the reactions; 2,5-bishydroxymethyltetrahydrofuran (**1**) and 1,2-dihydroxy-5,6-epoxyhexane (**2**).

When the inherent reactivity of epoxide groups leads to unavoidable cross-linking during epoxidation reaction, it may be necessary to look for other methods to utilize the modified starches for e.g. encapsulation. We and other investigators have shown that allyl starch derivatives can be synthesized in a mild a controllable way. It would be worth to investigate if these derivatives can be cross-linked for entrapment of certain bio-active molecules. This can be accomplished by reactions in which the double bonds are utilized, like ruthenium-catalyzed cross-alkene metathesis[27,28] or photopolymerization using dithiols.[29] However, if the reaction conditions applied during the epoxidation induce the premature cross-linking, an alternative method for epoxide formation should be sought after. For instance, the epoxidation of double bonds may be accomplished with a chemo-enzymatic approach. In this alternative route, *Candida antarctica* lipase B catalyzes the generation of peracid from a carboxylic acid, which is followed by a Prilezhaev epoxidation of the corresponding allyl ether under mild conditions.[30]

Since epoxy groups are very reactive towards nucleophiles such as amino and hydroxyl groups, epoxy starch derivatives are of interest for further functionalization with compounds having a coupling, complexing or cross-linking functions or compounds having hydrophobic, anionic or cationic groups (Figure 6.3). The production of these multifunctional starch derivatives may be used for various purposes such as delivery systems or controlled-release systems. Epoxy starch derivatives could be tested as carrier matrix for bioactive agents such as enzymes.



Figuur 3. Examples of functionalized epoxy starch derivatives. St: starch.

We performed some initial experiments on the entrapment of the thermostable β-glucosidase from *Pyrococcus furiosis* in epoxy starch derivatives, synthesized as described in Chapter 5. In these experiments, β-glucosidase was immobilized by mixing the thermostable enzyme with the pregelatinized epoxy-WMS under mild conditions (Table 6.3). The preliminary results clearly show that the irreversible binding of the enzyme to the gelatinized epoxy starch derivatives has been accomplished since 13% enzymatic activity recovery is found after fourfold extrusion of the immobilized β-glucosidase from the gelatinized epoxy starch derivatives. Nevertheless, a much higher binding efficiency may be achieved by optimization of the procedure. For this, it may be necessary to gain more detailed knowledge about the stability of β-glucosidase under the applied reaction conditions. Other improvements in the procedure might be found in variation of the ratio of amount of enzyme and carrier, and enhancing the interaction time of the enzyme with the pregelatinized starch.

Table 6.3. Enzymatic activity recovery of β-glucosidase by immobilization in pregelatinized epoxy-WMS.

β-glucosidase	Enzymatic activity (U) [c]	Recovery (%)
Free	25.0 ± 0.5	107
Free, incubated under immobilization conditions [a]	23.0 ± 0.4	100
Immobilized [a]	2.9 ± 0.5	13
Extruded from the starch gel [b] 1st step	15.4 ± 0.6	67
Extruded from the starch gel [b] 4th step	0.16 ± 0.01	0.7

[a] Immobilization conditions: incubation of β-glucosidase (0.05 mg, 0.9 mg mL^{-1}) with or without pregelatinized epoxy-WMS (50 mg; 60 min at 80°C) in 1.0 mL 50 mM citrate buffer, pH 5.0 at 80°C for 30 min. [b] Extrusion, 1-4 steps: the starch gel was suspended in 0.5 mL citrate buffer followed by centrifugation at 12,000 rpm, for 10 min. In total, 83% of enzyme activity was recovered by the extrusions. [c] Measured in an enzymatic activity assay for β-glucosidase as described elswhere.[31] One unit of enzyme activity (U) was defined as the amount of enzyme catalyzing the liberation of 1.0 μmol of *p*-nitrophenol per min at 50°C.

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List of abbreviations and symbols

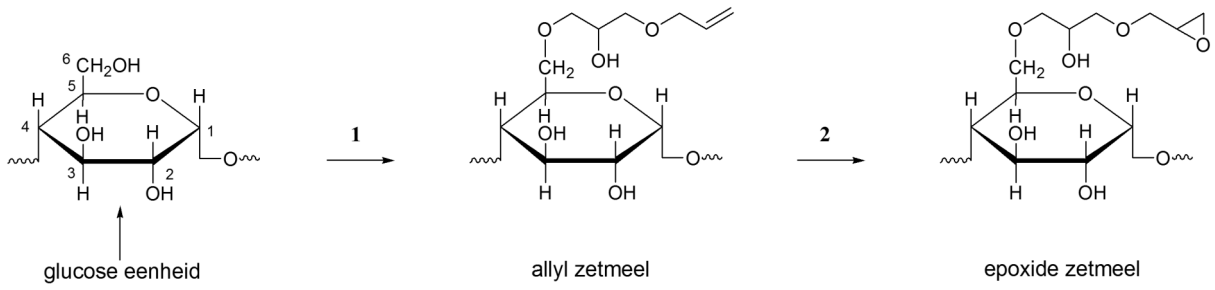
AEMS	amylose-enriched maize starch
AGE	allyl glycidyl ether
AGU	anhydrous glucose unit
AHP	1-allyloxy-2-hydroxypropyl
a_w	water activity
C_{starch}	starch suspension
CCD	central composite experimental design
COSY	^{1}H-^{1}H correlation NMR spectroscopy
Cr	cross-link
DEPT	distortionless enhancement by polarization transfer
DL	diol group
DMSO	dimethyl sulfoxide
DP	degree of polymerization
ds	dry starch
DS	degree of substitution
DSC	differential scanning calorimetry
eff.	efficiency
eq.	equation
FFD	fractional factorial design
GC-MS	capillary gas chromatography-mass spectroscopy
ΔH_{gel}	enthalpie of gelatinization
HD	1,6-hexanediamine
HETCORR	^{1}H-^{13}C heteronuclear chemical shift correlation
HMBC	^{1}H-^{13}C heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion chromatography
HPAEC	high performance anion exchange chromatography
I	integrated peak area
IR	infrared
L	droplet length
MA	maleic acid
MALDI-TOF MS	matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

MG	methyl α-D-glucopyranoside
MS	dent maize starch
m/z	mass over charge ratio
MW	molecular weight
n	moles
*p*NBP	4-(4-nitrobenzyl pyridine)
n.d.	not determined
NMR	nuclear magnetic resonance
Ox	epoxy group
p	viscosity ratio of the droplet and matrix
PAD	pulsed amperometric detection
PDMS	polydimethylsiloxane
R^2	determination coefficient
RH	relative humidity
RVA	rapid visco-analyser
RVU	RVA unit
s.u.	strain units
SEM	scanning electron microscopy
T	rotation period
$T_{pasting}$	pasting (onset) temperature
T_{peak}	peak temperature
T_c	completion temperature
T_o	onset temperature
T_p	peak temperature
TMS	tetramethylsilane
WMS	waxy maize starch
XRD	X-ray diffraction
UV	ultraviolet
Vis	visible
w/w	weight fraction
x	molarity
γ	shear rate
δ	chemical shift
θ	angle (X ray diffraction)
σ	shear stress

Samenvatting

Zetmeel is een natuurlijk polymeer, dat voornamelijk is opgebouwd uit aaneengesloten α-(1,4)-glucose eenheden. Het bestaat uit twee structuurvormen, amylose en amylopectine. Vanwege de gunstige eigenschappen zoals biologische afbreekbaarheid, lage prijs, grote beschikbaarheid en gemakkelijke winbaarheid uit verschillende zetmeelbronnen (maïs, aardappelen), is zetmeel een uitstekend materiaal voor industrieel gebruik. Zo wordt zetmeel veelvuldig toegepast in voedingsmiddelen, papier, textiel en in de farmaceutische industrie. Om de functionaliteit te verbeteren en te voldoen aan de industriële behoeften wordt zetmeel vaak enzymatisch, fysisch of chemisch gemodificeerd. De introductie van een functionele groep in het biopolymeer leidt tot duidelijke veranderingen in zetmeeleigenschappen, afhankelijk van de functionele groep en de substitutiegraad. Door deze chemische modificaties veranderen de specifieke eigenschappen, zoals lagere verstijfseling, verbeterde stabiliteit bij koude opslag en aangepaste visco-elastische eigenschappen. De meeste commerciële zetmeelderivaten hebben een substitutiegraad oplopend tot 0.2. Ze worden breed toegepast in industriële producten als afgiftesystemen voor geneesmiddelen en als stabilisatoren en bindingsmiddelen in voedingsmiddelen. Een algemene beschrijving van de structuur van zetmeel, de fysisch-chemische eigenschappen van zetmeel en een aantal chemische modificaties van zetmeel zijn samengevat in *hoofdstuk 1*.

Vanuit de farmaceutische industrie is er grote belangstelling naar korrelachtige multifunctionele zetmeelderivaten, die gebruikt kunnen worden als biologisch afbreekbare afgiftesystemen. De productie van deze multifunctionele zetmelen vereist een functionele groep aan het zetmeel die kan binden met een scala van andere stoffen (liganden). Epoxide zetmeelderivaten behoren tot deze groep van gemodificeerde zetmeelproducten. Epoxidegroepen zijn actieve groepen en reageren snel met nucleofiele liganden die amino- en hydroxylgroepen bevatten zoals peptiden, aminozuren en polyolen. Bovendien kunnen epoxidegroepen verbindingen maken met de hydroxylgroepen in het zetmeel waardoor een matrix gevormd wordt in het zetmeel. De gevormde matrices kunnen gebruikt worden voor het fysisch inkapselen van chemische stoffen zoals insecticiden en bioactieve stoffen, bijvoorbeeld enzymen.



Figuur 1. Synthetische route van epoxide zetmeelderivaten. Zetmeel is gemodificeerd met allyl glycidyl ether (**1**) gevolgd door epoxidatie van de dubbele band (**2**).

Het doel van het onderzoek beschreven in dit proefschrift is het ontwikkelen en het optimaliseren van een mild en milieuvriendelijk proces voor de productie van reactieve, korrelachtige epoxide zetmeelderivaten (Figuur 1). Via een tweestaps reactie is zetmeel eerst gemodificeerd met een reagens die een allylgroep bevat (allyl glycidyl ether) en vervolgens is de dubbele band geëpoxideerd. Verschillende studies zijn uitgevoerd om meer inzicht te krijgen in het mechanisme van zetmeelmodificatie en in de factoren die de reactie beïnvloeden. Daarbij is gebruik gemaakt van een specifieke methode om de procescondities van de eerste stap in de synthese te optimaliseren (Ontwerp van Experimenten). Verder zijn de eigenschappen en het gedrag van het gemodificeerd zetmeel op moleculair en macroscopisch niveau bestudeerd.

Multifunctionele zetmeelderivaten

In *hoofdstuk 2* van dit proefschrift wordt de eerste stap van de zetmeelmodificatie voor de ontwikkeling van epoxide zetmeelderivaten beschreven. In deze studie is een milde reactie toegepast waarin waxy maïszetmeel (hoofdzakelijk amylopectine) en amylose verrijkt maïszetmeel (bevat 70% amylose en 30% amylopectine) gemodificeerd zijn met allyl glycidyl ether (Figuur 1, stap **1**). De chemische modificatie vond plaats in een verdunde alkalische oplossing onder de verstijfselingstemperaturen van de maïszetmelen. Het product, 1-allyloxy-2-hydroxypropyl-(AHP)-zetmeel (allyl zetmeel), kon daardoor eenvoudig via filtratie geïsoleerd worden. Met behulp van proton nucleaire magnetische resonantie (^{1}H NMR) werd een substitutiegraad (degree of substitution, DS) van ± 0,20 voor beide zetmelen bepaald. Dit geeft aan dat de waxy en amylose verrijkte

maïszetmeelkorrels goed toegankelijk zijn voor het reagens onder de heterogene reactiecondities.

Het mechanisme van de zetmeelmodificatie is verder bestudeerd door de positie van allylgroepen op de glucose eenheid en de distributie van allylgroepen over de zetmeel-moleculen te onderzoeken. 1D en 2D NMR metingen suggereerden dat de glucose eenheid in het zetmeel voornamelijk gesubstitueerd is op de O-6 positie. Deze informatie is naderhand gevalideerd met een gemodificeerd monosaccharide (methyl-α-glucopyranoside), die als modelstructuur voor een glucose eenheid in zetmeel is genomen. Vervolgens is de distributie van allyl glycidyl ether over de amylose en de amylopectine van de gemodificeerde zetmelen bestudeerd met behulp van enzymatische afbraak gevolgd door karakterisering van de verkregen fragmenten met scheidingstechnieken zoals Matrix-Assisted Laser Desorption/ Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS). De gebruikte enzymen bleken gehinderd te worden door de allylgroepen in gemodificeerd amylose verrijkt zetmeel (bevat 70% amylose) en waxy maïszetmeel (voornamelijk amylopectine). De verkregen fragmenten van de gemodificeerde zetmelen gaven significante verschillen in de grootte en substitutiepatroon in de MALDI-TOF MS analyse. Het gemodificeerde amylose verrijkt zetmeel toonde een heterogene distributie van allylgroepen over de polymeren, dat wil zeggen meer fragmenten en grotere fragmenten met geclusterde groepen en een hoge substitutiegraad. Het gemodificeerde waxy maïszetmeel daarentegen gaf een homogene distributie van de allylgroepen over het polymeer. Gezien deze verschillen in de substitutiepatronen van amylose verrijkt en amylopectine-houdende zetmeel, is er aangenomen dat de substitutie voornamelijk in het amorfe gebied van de zetmeelkorrel heeft plaatsgevonden.

In *hoofdstuk 3* wordt de optimalisatie van de procescondities voor de synthese van allyl waxy en dent maïszetmeel onderzocht. Daarbij is er gebruik gemaakt van een specifieke methode (Statistisch Ontwerp van Experimenten) die het mogelijk maakt maximale informatie over de procescondities te verkrijgen uit een minimaal aantal experimenten. Het doel van deze studie was om het effect van de procescondities op de substitutiegraad van allyl zetmeelderivaten te bepalen. In twee stappen zijn de meeste belangrijk procescondities bepaald en vervolgens is de reactie geoptimaliseerd met behulp van deze significante factoren.

In de onderzoeksfase zijn 35 experimenten gegenereerd uit zes geselecteerde reactiefactoren. Na uitvoering van de experimenten en analyse van de producten bleek uit statistische studies dat de temperatuur het voornaamste effect op de DS van waxy en dent maïszetmeel had. Voor het uiteindelijk optimalisatiemodel is de reproduceerbaarheid van het model verder onderzocht. In tweede fase zijn vier variabelen verder geëvalueerd met behulp van 25 toegevoegde experimenten. Uit de statistische analyse van de resultaten bleek de temperatuur weer de meest belangrijke procesconditie in de modificatie van waxy en dent maïszetmeel te zijn. Daarnaast hadden de natriumhydroxide en allyl glycidyl ether concentraties evenzeer een significant effect op de DS. Nadien zijn deze gegevens gebruikt voor de optimalisatie van de reactie. Daaruit bleek dat er grote verschillen zijn in maximale DS en omzettingsefficiëntie tussen de optimale condities voor waxy en dent maïszetmeel. Deze verschillen kunnen worden verklaard door het verschil in de hoeveelheid allyl glycidyl ether die nodig is voor de synthese, en de toegankelijkheid van het zetmeel voor allyl glycidyl ether.

In *hoofdstuk 4* worden de eigenschappen en gedrag van gemodificeerd waxy, dent en amylose verrijkt maïszetmeel beschreven. Door de verethering van het zetmeel zijn de eigenschappen en gedrag van zetmeel veranderd. Zo bleek dat de zwelling en de oplosbaarheid van zetmeelkorrels toeneemt na reactie met allyl glycidyl ether. Verder werd er waargenomen dat de zwellingskracht afnam naarmate er meer amylose in het gemodificeerd zetmeel aanwezig was (allyl waxy > allyl dent > allyl amylose verrijkt maïszetmeel). Bovendien bleek dat de zwellingskracht en oplosbaarheidsindex van gemodificeerd waxy en dent maïszetmeel toenam met toenemende DS tot een verzadigingsgrens. Omdat de zwellingscapaciteit bij kamertemperatuur al hoog is, vindt er vermoedelijk maar een kleine verandering plaats in de zwellingscapaciteit voor gemodificeerd zetmeel bij een hogere temperatuur. Als gevolg van toenemende zwelling van de zetmeelkorrels werden er verlaagde verstijfselingstemperaturen voor de gemodificeerde zetmelen gevonden in vergelijking met de natuurlijk zetmelen. Deze verschillen worden veroorzaakt door de invloed van allylgroepen op de interacties tussen de zetmeelmoleculen. Verder leidden de structuurveranderingen in de zetmeelkorrels ook tot verandering in viscositeit van zetmeeloplossingen. De viscositeit van gemodificeerd waxy en dent maïszetmeel oplossingen nam af tijdens verwarming en vertoonde gelachtige eigenschappen tijdens afkoeling voor beide zetmelen.

Met behulp van reologische en reo-optische studies is het kwalitatieve verschil in de stroombeweging van gemodificeerde en natuurlijke zetmeeloplossingen onderzocht. Druppels met maximaal gezwollen korrels van gemodificeerd waxy en dent maïszetmeel en niet-gemodificeerde zetmeeloplossingen zijn bestudeerd door deze druppels te plaatsen in een niet vervormbare matrixvloeistof en te bekijken onder een optische microscoop. Door de toenemende stroming in de matrix verlengden en verdeelden de druppels van de gemodificeerde zetmeeloplossingen zich in verscheidene kleinere druppels. Daarentegen vertoonden de druppels van de natuurlijke zetmeeloplossingen geen scheiding of een verlenging. Ze vormde alleen een "staart", of draaide rond in de rotatierichting. Het verschil in druppelgedrag tussen gemodificeerde en natuurlijke zetmeeloplossingen worden veroorzaakt door het verschil in zwellingskracht van de zetmeelkorrels die gerelateerd is aan de polymerenconcentratie en de korrelvorm, en dus aan de viscositeit van de oplossing. Het druppelgedrag van de gemodificeerde zetmeeloplossingen is te vergelijken met een vloeibare druppel. Het niet scheiden van de druppels van de natuurlijke zetmeeloplossing kan mogelijk worden verklaard door de aanwezigheid van water tussen de gezwollen korrels, hetgeen vervorming blokkeert.

Tenslotte is de epoxidatie van de dubbele band van allyl zetmeel bestudeerd (*hoofdstuk 5*). De productie van epoxide zetmeelderivaten (Figuur 1, stap **2**) is succesvol uitgevoerd met allyl waxy maïszetmeel en waterstofperoxide in combinatie met acetonitril. Met een zelfontwikkelde tweestaps spectrofotometrische methode werd bepaald dat maar 11% van de dubbele banden in het zetmeel in een epoxidegroep omgezet was. Een hogere epoxidatiegraad was verwacht, maar de epoxidegroepen bleken niet stabiel te zijn onder de gebruikte reactiecondities. Dit bleek uit de gereduceerde zwellingskracht en oplosbaarheidindex van het epoxide zetmeelderivaat, die wijzen naar verknopingen in het zetmeel. De structuurveranderingen zijn vervolgens onderzocht door de allyl en epoxide zetmeelderivaten enzymatisch te hydrolyseren en vervolgens de verkregen fragmenten te analyseren met MALDI-TOF MS. In het substitutiepatroon van het geëpoxideerde derivaat werden enkele kleine fragmenten met allyl en epoxidegroepen aangetroffen. Verder werden er diverse andere fragmenten gevonden die vermoedelijk gevormd zijn door vervolgreacties van epoxide-groepen, zoals verknopingen in het zetmeel of hydratatie van de epoxidegroepen.

Toekomstperspectieven en alternatieve benaderingen

In *hoofdstuk 6* worden de resultaten van dit proefschrift bediscussieerd en worden enkele toekomstperspectieven voor het onderzoek naar multifunctionele zetmeelderivaten toegelicht. In principe zijn de experimenten beschreven in hoofdstuk 2, 3 en 4 modelstudies voor modificerende stoffen die vergelijkbaar zijn met allyl glycidyl ether en/of een langere ketenlengte bevatten. Naast de veranderingen in de eigenschappen van gemodificeerd zetmeel kunnen de modificerende stoffen met een langere ketenlengte eventueel leiden tot minder verknopingen en dus een hogere epoxidatiegraad.

Ofschoon de in deze studie beschreven epoxidatiestap succesvol was, bevatte het epoxide zetmeelderivaat een lage concentratie epoxidegroepen, vanwege vervolgreacties van de epoxidegroepen. Dit is de reden dat er verder gezocht is naar reacties die een hogere epoxidatiegraad van zetmeel zouden kunnen opleveren. De meest uitgewerkte studie is een systeem waarin diepoxides zijn gebruikt voor het epoxideren van methyl-α-glucopyranoside (modelstructuur). Uit deze studie is geconcludeerd dat diepoxides liever met zichzelf dan met ander stoffen reageren. Een andere optie is de epoxidatie van allyl zetmeelderivaten uit te voeren via een chemoenzymatische reactie, als er vanuit gegaan wordt dat de verknopingen in zetmeel vooral het gevolg zijn van de gebruikte reactiecondities.

Aangezien epoxidegroepen snel reageren met nucleofiele groepen kan het interessant zijn epoxide zetmeelderivaten verder te functionaliseren met stoffen die bijvoorbeeld kunnen koppelen, geladen zijn of verknopingen in het zetmeel vormen. Voor toepassingen van deze multifunctionele zetmeelderivaten kan gedacht worden aan onder andere afgiftesystemen of matrices waaruit stoffen gecontroleerd vrijkomen. Epoxide zetmeelderivaten kunnen bijvoorbeeld toegepast worden als drager voor bioactieve reagentia zoals enzymen. Ter illustratie is er een korte studie uitgevoerd waarin het epoxide zetmeelderivaat (hoofdstuk 5) gebruikt is als matrix voor het immobiliseren van een thermofiel β-glucosidase. Uit de resultaten werd geconcludeerd dat β-glucosidase irreversibel gebonden was aan het epoxide zetmeelderivaat, zij het in lage efficiëntie (13%). Verder onderzoek is nodig voor de optimalisatie van deze toepassing en voor andere toekomstige applicaties.

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Annemarie

Curriculum Vitae

Anna Maria Leontina Huijbrechts, roepnaam Annemarie, werd op 9 oktober 1978 geboren te Breda. In 1997 behaalde zij haar VWO diploma aan het Mencia de Mendoza Lyceum, eveneens in Breda. In hetzelfde jaar begon zij met de studie Voeding en Diëtetiek aan de Haagse Hogeschool te ’s-Gravenhage. Na het afronden van het propedeusejaar van deze opleiding, begon ze in augustus 1998 met de studie Moleculaire Wetenschappen aan Wageningen Universiteit. Tijdens deze studie heeft ze zowel een afstudeervak gedaan bij het Laboratorium voor Organische Chemie van Wageningen Universiteit, als bij het departement Radiologie van het Universitair Medisch Centrum St. Radboud te Nijmegen. Daarna heeft ze de studie afgerond met een stage aan de University of Oxford (UK) en in 2003 haar doctoraal diploma behaald. Aansluitend startte zij haar promotieonderzoek, dat beschreven is in dit proefschrift. Het promotieonderzoek is uitgevoerd bij het Laboratorium voor Organische Chemie van Wageningen Universiteit en Agrotechnology & Food Sciences Group.

List of publications

A.M.L. Huijbrechts, G.M. Visser, C.G. Boeriu, E.J.R. Sudhölter. Starch-based multifunctional epoxy starch derivatives for immobilization of bioactive molecules. *Chemistry meets Biology*, *Proceedings of FEBS Advanced Course* (2005) 19, Island of Spetses, Greece.

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Overview of completed training activities

Discipline specific activities

Courses

8th European training course on carbohydrate 'Summer Course Glycosciences', VLAG	2004
IASOC '04 on 'Creativity in Organic Synthesis from Target to Function', Italy	2004
FEBS advanced course on 'Chemistry Meets Biology', Greece	2005
7th International symposium on 'Biocatalysis and Biotransformations' in Delft, Biotrans	2005
EPNOE mobility for 'Counter rotation measurements', Centre for Material Forming (CEMEF) in Sophia-Antipolis, France	2006
2nd International advanced course of 'Bio-nanotechnology', VLAG	2006
EPNOE workshop on 'Working Conditions and Gender Equality' in Lodz, Poland	2006

Meetings

NWO meeting of the study groups 'Design and Synthesis', 'Structure and Reactivity', 'Biomolecular Chemistry' in Lunteren	2004-2007
44th Thematic meeting on 'Enzymatic Conversion of Biopolymers', VLAG	2005
Symposium Bio-nanotechnology on 'Assembly and Nanostructures', WUR	2005
EPNOE Scientific Meeting in Iaşi, Romania	2007
59th International Starch Convention, Association of Cereal Research in Detmold, Germany	2008

General courses

Organizing and supervising thesis work, OWU	2004
Project planning and time management, WGS	2004
Scientific writing, CENTA, WUR	2005
Manifestation 'Het Element' for molecular science organized by the KNCV	2005
PhD Competence assessment, WGS	2006
Postgraduate course on 'Basic and Advanced Statistics', PE & CR	2007

Optionals

Preparing PhD research proposal, VLAG	
Ph.D. Study tour, Laboratory of Organic Chemistry, USA	2005
Annual meetings of Carbohydrate Research Center-Wageningen, WUR	2003-2007
Laboratory of Organic Chemistry Colloquia, WUR	2003-2008

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