Starch structures and their usefulness in the production of packaging materials

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Abstract

Environmental concerns about increasing industrial use of plastics and the associated waste are raising the demand for renewable sources to replace petroleum-based products, especially in the packaging sector. This thesis examined the relationship between molecular structures of starch and their material properties in coating materials for food packaging. Chemical modification of starch with citric acid and tailored starches from genetically modified potatoes with higher amylose content was used to improve film formation, reduce water sensitivity and decrease gas permeation.

Citric acid was used as a cross-linking agent and as a plasticiser to improve the barrier properties of starch-based barrier coatings. Methods to detect the cross-linking of starch by quantifying the di-ester content of citric acid and measuring molecular weight changes were developed. Starch films containing highest citric acid content showed the highest cross-linking density and lowest water solubility, but also showed the highest degree of starch degradation due to acid hydrolysis. Adjustment of pH in the starch formulation was used to control starch degradation and adapt it to industrial needs. At pH 4, hydrolysis of starch nearly stopped but cross-linking reaction still occurred, leading to minimum gas permeation. Cross-linking occurred already at temperatures as low as 70°C. Hence no curing step was needed to initiate cross-linking reaction. This is suitable for industrial paper coating applications since high temperature is not required.

The coating process had a great impact on the molecular structure of starch. Laboratory-scale coatings showed a lower degree of cross-linking and no significant hydrolysis of starch compared with solution-cast films, a difference which could be attributable to shorter drying time in laboratory-scale coatings. The high evaporation rate in industrial pilot-scale coatings promoted cross-linking, but also increased coating surface unevenness, resulting in pinholes, which diminished barrier properties.

Studies of tailored starches from genetically modified potatoes with expected increased amylose content showed irregular granules but no weakening of birefringence. It was found that altered amylopectin structure rather than high amylose content resulted in better barrier properties. The amylose-like structure formed in the cast films of starches from genetically modified potatoes decreased oxygen permeability and improved film strength.

Keywords: potato starch, film formation, amylose, amylopectin, citric acid, cross-linking, hydrolysis, molecular weight, barrier properties, coatings, biopolymers

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Menzel C***, Olsson E*, Plivelic TS, Andersson R, Johansson C, Kuktaite R, Järnström L, Koch K (2013). Molecular structure of citric acid cross-linked starch films. *Carbohydrate Polymers* 96, 270-276.

II Olsson E*, **Menzel C*,** Johansson C, Andersson R, Koch K, Järnström L (2013). The effect of pH on hydrolysis, cross-linking and barrier properties of starch barriers containing citric acid. *Carbohydrate Polymers*, 98, 1505-1513.

III **Menzel C,** Koch K (2014) Impact of the coating process on the molecular structure of starch-based barrier coatings. *Journal of Applied Polymer Science* (in press, doi: 10.1002/app.41190).

IV **Menzel C**, Andersson M, Andersson R, Vázquez-Gutiérrez J L, Daniel G, Langton M, Gällstedt M, Koch K. The molecular structure of high-amylose potato starches and their material properties (manuscript).

Papers I-III were reproduced with the permission of the publishers.

* First authorship shared

The contribution of Carolin Menzel to the papers included in this thesis was as follows:

I Contributed equally with Erik Olsson. Participated in planning the experiments and evaluation of results, performed half the experiments and had major responsibility for writing and revising the manuscript.

II Contributed equally with Erik Olsson. Participated in planning the experiments and evaluation of results, performed half the experiments and writing revision of the manuscript.

III Participated in planning the study and evaluation of results. Was responsible for laboratory analyses and for writing and revising the manuscript.

IV Participated in planning the experiments and evaluation of results. Was responsible for laboratory analyses and for writing the manuscript.

Related work by the same author not included in this thesis

Muneer F, Andersson M, Koch K, **Menzel C**, Hedenqvist M, Gällstedt M, Plivelic T, Kuktaite R. Nano-structural conformation of plasticized wheat gluten and novel potato starch composites and its relation to mechanical and barrier properties (manuscript).

Andersson R, Johansson C, Järnström L, Koch K, **Menzel C,** Olsson E
A fiber-based substrate provided with a coating based on biopolymer material and a method of producing it. SE-1250568-1, Swedish patent application, filed 31 May 2012

1 Implications

The work presented in this thesis forms part of two national projects aiming to use renewable resources as functional barriers in food packaging materials. Within the interdisciplinary collaboration *Renewable Functional Barriers*, industry and academia sought to replace synthetic polymers with *e.g*. potato starch. To produce coatings with good barrier properties and lower sensitivity to water, citric acid was used as a cross-linking agent and plasticiser. All process steps were investigated, in studies ranging from laboratory-scale to pilot-scale trials, and different process parameters were studied.

A different approach was used in the project *Trees and Crops for the Future*, where tailored starches were studied as barrier coatings. Potatoes with increased amylose content produced by biotechnological tools were tested for use in enhancing the film formation and barrier properties. Designing functionality within the crop requires less input for post-harvest modifications, and hence decreased environmental and economic impacts.

1.1 Potential of starch as bio-based material for food packaging

Starch is the most abundant reserve polysaccharide in plants. Today, the main sources of starch extraction are tubers, roots and seeds, primarily from maize, tapioca, potato, wheat and rice. Starch can easily be extracted with high purity, resulting in a white, tasteless and odourless powder. These good organoleptic properties makes it an interesting resource for manifold applications, not only in human food and animal feed, but also as feedstock for non-food industrial applications such as pulp and paper, adhesives and bioethanol. Starch is also biodegradable and can exhibit thermoplastic behaviour. In 2010, starch represented 22.2% of the global bioplastic packaging market (Pierce, 2011). With growing demand for food packaging of 2.5% *per annum* (Reportlinker), the production capacity for bioplastics is predicted to increase fivefold from

2011 to 2016 (*EuBP market data*, 2012). Starch is one of the promising biopolymers to replace commercial plastics and hence has been widely studied since the 1950`s (Wolff *et al.*, 1951).

However, the applications of starch as a film and coating in food packaging are limited, due to its water sensitivity and poor mechanical and water vapour barrier properties. Several chemical modifications have been used over the years in an attempt to overcome the hydrophilic character of wild-type starch. Commonly used plasticisers are glycerol and low-molecular weight polyhydroxy compounds (Lourdin *et al.*, 1997).

In the paper industry, oxidation of starch is commonly used to lower the viscosity by reducing molecular weight and lowering the gelatinisation temperature of starch. In addition, hydroxypropylation of starch has been shown to improve film formation in terms of flexibility, low-temperature stability and beneficial rheological properties (Jansson & Järnström, 2005).

1.2 Starch granules and molecular structure of starch components

Starch consists solely of glucose residues that are linked in two different forms, building up the two polymers amylose and amylopectin. Amylose is basically a linear chain with α-1,4 linkages, whereas in amylopectin glucose residues are also joined by α-1,6 linkages. The highly branched structure results in a polymer with a molecular weight of 10^7-10^9 Da, while linear amylose has a lower molecular weight of 10^5-10^6 Da. Amylopectin is the main component in starch, comprising 70-80%. Both polymers are systematically ordered in granules. Granule size and shape differ depending on starch source. In potato, starch granules are oval with a wide size ranging from 5 to 100 µm. Starch granules are semi-crystalline, with alternating amorphous and semi-crystalline zones which build up growth rings (Figure 1). At a higher organisation level, spherical blocklet-like structures have been shown (Gallant *et al.*, 1997). Each blocklet contains several amorphous and crystalline lamellae with a periodic spacing of about 9 nm in potato (Zobel, 1988). The crystalline lamellae are built up of double helices of the outer chains of the amylopectin, whereas the amorphous lamellae consist of the branch points and inner chains.

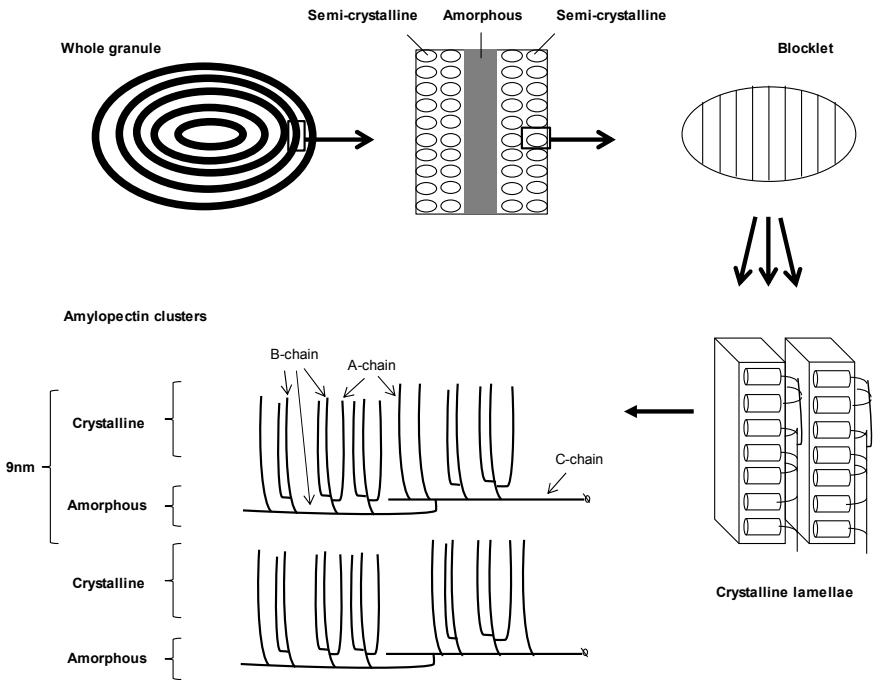


Figure 1. Schematic illustration of starch granule organisation showing the occurrence of alternating growth rings, blocklet structures and double helices in crystalline lamellae [adapted from Gallant *et al.* (1997) and Pérez and Bertoft (2010)].

The location of amylose within the starch granule is still not clear and different hypotheses have been suggested. One of these suggests the randomly interspersed location of individual chains among the amylopectin clusters in the semi-crystalline and amorphous regions, with enrichment of amylose towards the periphery of the granule (Kasemsuwan & Jane, 1994; Jane & Shen, 1993). In high-amylose starch granules, amylose has been shown to be concentrated in the hilum of the granule (Blennow *et al.*, 2003). The majority of amylose molecules are reported to be in the single helical state (Ring *et al.*, 1985), and only some larger molecules are involved in double helices with amylopectin (Kasemsuwan & Jane, 1994).

The structure of amylopectin molecules is based on three types of glucose chains, A-, B- and C-chains (Peat *et al.*, 1952). Each amylopectin molecule consists of a single C-chain carrying one reducing end. B-chains are connected by their reducing end to the C-chain and/or to other B-chains. A-chains are the outermost chains and carry no chain themselves. Gel permeation chromatography of amylopectin molecules after debranching with isoamylase

and pullulanase (hydrolysing 1-6 linkages) reveals the distribution of chains into different groups. Short chains, with chain length from 6 to 17 glucose residues, have been shown to be characteristic for amylopectin from different samples (Koizumi *et al.*, 1991). Chains in amylopectin are organised in clusters where short chains constitute the clusters while longer chains of DP>35 interconnect them (Hizukuri, 1986).

1.3 Starch modifications

Even though starches offer unique properties for various applications, wild-type starch suffers from its high tendency to retrograde, thermal decomposition and low shear resistance (Hoover, 2001). To overcome these limitations, the structure of starch can be altered through physical treatment, chemical derivatisation or decomposition methods such as hydrolysis and oxidation. Modified starches can be used for manifold applications.

In this thesis chemical and genetic modifications were used to enhance starch functionality. These modifications are described in more detail below. However, physical and enzyme-catalysed modifications are also used in industry, but those are not discussed in detail at this point.

1.3.1 Chemical modifications/ derivatisation

Starch consists of glucose residues, so there are three hydroxyl groups, on each glucose residue at carbon position 2, 3 and 6, that can undergo chemical reactions. Chemical modifications involve the inclusion of a functional group. The type and number of functional groups introduced into the starch molecule depend on the required property in the end product. The final functionality depends on the reaction conditions, the starch source, the amount and type of substituent and its distribution along the molecule (Singh *et al.*, 2007). Common derivatisations are esterification, etherification, cross-linking and dual modifications.

The introduction of a hydroxypropyl group into starch has been shown to enhance its cold-water solubility, improve its shear resistance and stabilise its viscosity (Jarowenko, 1978). Hydroxypropyl-starches are widely used in the food industry in products such as puddings, sauces and dips because of their increased clarity and improved freeze-thaw and cold storage stability. This type of modified starch is also suitable in barrier coatings because of its high transparency (Vorwerg *et al.*, 2004), better film formation and good rheological properties (Jansson & Järnström, 2005).

Typical esterification reactions are acetylation and the introduction of a fatty acid chain to lower gelatinisation temperature and the tendency to form

gels or to retrograde (Chi *et al.*, 2008; Shogren, 2003; Lohmar & Rist, 1950). Low acetylated and highly acetylated starches show different properties, *e.g*. low-substituted starches are used as a film former, thickener or stabiliser, whereas high-substituted starch acetates are used as coatings in the paper industry (Koch *et al.*, 2014; Haasmaa *et al.*, 2003; Neigel *et al.*, 1995) and for drug release products (Tarvainen *et al.*, 2004).

Decomposition of starch by introducing carboxyl and carbonyl groups through oxidation or hydrolysis is a common principle to produce starches with lower viscosity. These groups can act as an internal plasticiser and can prevent the tendency to retrograde. They are used in the paper industry to increase the solid content in formulation for starch coatings, since less water has to be evaporated (Wurzburg, 1986a).

Cross-linking of starch is intended to form bonds between one polymer chain and another, inter- and intra-molecularly, and to strengthen the material. Cross-links are generated by treatment with multifunctional reagents that are capable of forming ester or ether bonds. The most commonly used reagents include epichlorohydrin, phosphoryl chloride, ortho-phosphoric acid and their salts, and many di- or multi-carboxylic acids (Ashogbon & Akintayo, 2014; Kaur *et al.*, 2012; Singh *et al.*, 2007; Woo & Seib, 2002). Cross-links can be initiated by chemical reaction through heat, pressure, pH or radiation. Covalent cross-links are mechanically and thermally stable, *e.g*. starch pastes made from cross-linked starch granules are more stable to swelling, high temperature and shear and acidic conditions (Wurzburg, 1986b). They are used as texturisers in soups and sauces, and in bakery and dairy products to improve cooking properties (Hirsch & Kokini, 2002). In addition, cross-linking has been shown to reduce the water sensitivity of starch and to improve barrier properties in starch-based coatings (Olsson *et al.*, 2013; Ghanbarzadeh *et al.*, 2011; Miller & Krochta, 1997).

1.3.2 Reactions of starch with citric acid – esterification, cross-linking, plasticisation and hydrolysis

Citric acid is an organic acid with three carboxyl groups and one hydroxyl group (Figure 2). Hence, it can react with the hydroxyl groups in starch to create esters, which can result in mono-, di- and tri-esters (Klaushofer & Bleier, 1982; Gramera *et al.*, 1966). The reaction is a typical Fischer esterification reaction, which can be catalysed by reducing the pH or adding Lewis acids, as shown in Figure 2. Cross-linking and di-esterification (Figure 3) is a two-stage esterification process that has been shown to be promoted by high temperature treatment at temperatures well above 100 °C (Ghosh Dastidar

& Netravali, 2012; Olivato *et al.*, 2012; Shi *et al.*, 2007; Wang *et al.*, 2007; Wing, 1996).

It has been shown that highly and weakly substituted regions occur in starch and it has been suggested that esterification of starch with citric acid mainly takes place around the branching points of amylopectin (Klaushofer & Bleier, 1983). The formation of tri-esters is doubtful (Bleier & Klaushofer, 1983).

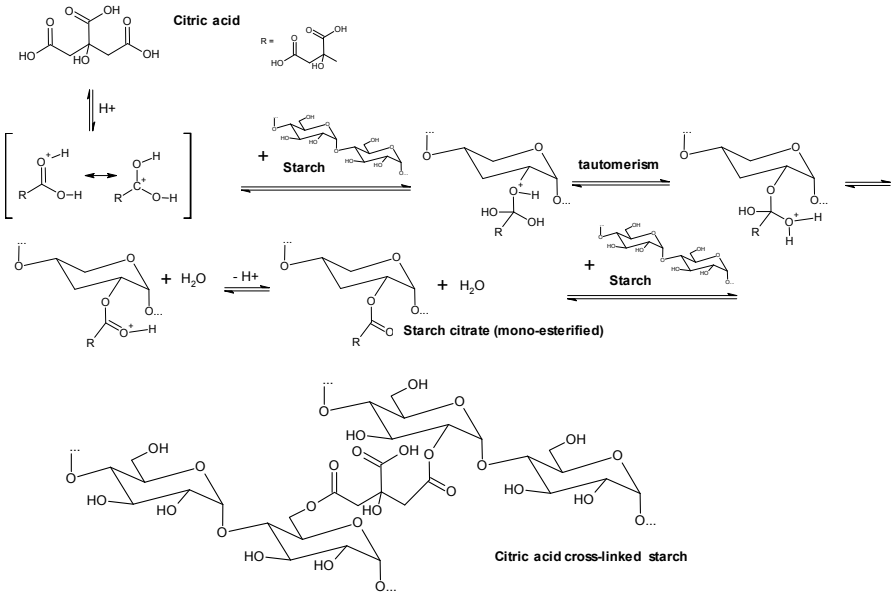


Figure 2. Schematic illustration of the acid-catalysed esterification of citric acid and starch to starch citrate (mono-esterified) and a possible structure of citric acid cross-linked starch (di-esterified).

Mono-esterified starch is beneficial since it can act as an internal plasticiser of starch by disrupting inter- and intra-molecular hydrogen bonds. On the other hand, unreacted 'free' citric acid can act as an external plasticiser and increase the flexibility of the starch polymers in films and coatings (Ghanbarzadeh *et al.*, 2011; Reddy & Yang, 2010; Shi *et al.*, 2007).

In cases where two carboxyl groups of citric acid are esterified, intra-molecular bonds within the same polymer, or inter-molecular bonds between two polymers are formed. Bleier and Klaushofer (1983) have shown that intra-molecular di-esters can occur between two neighbouring glucose residues, between two glucose chains in the same polymer or even within the same glucose residue on C6- and C3-position. However, only inter-molecular di-esters, which result in an increase in molecular weight, are considered to be

cross-linkages in this thesis. Cross-linking is desirable since it reduces swelling and solubility in water and increases viscosity.

A concurrent reaction to esterification of starch by citric acid is hydrolysis. Even though citric acid is a weak organic acid, starch can easily be hydrolysed due to the low pH at high temperature. It has been shown that increasing citric acid concentration and high temperature promote degradation of starch (Shi *et al.*, 2007; Carvalho *et al.*, 2005; Hirashima *et al.*, 2004; Wing, 1996). During hydrolysis, the glycosidic oxygen is protonated and a water molecule is added, to yield the reducing sugar group. Hence, molecular weight is reduced, which in turn increases diffusion and permeability in starch films. This in turn increases the molecular movement, which is undesirable in the paper industry.

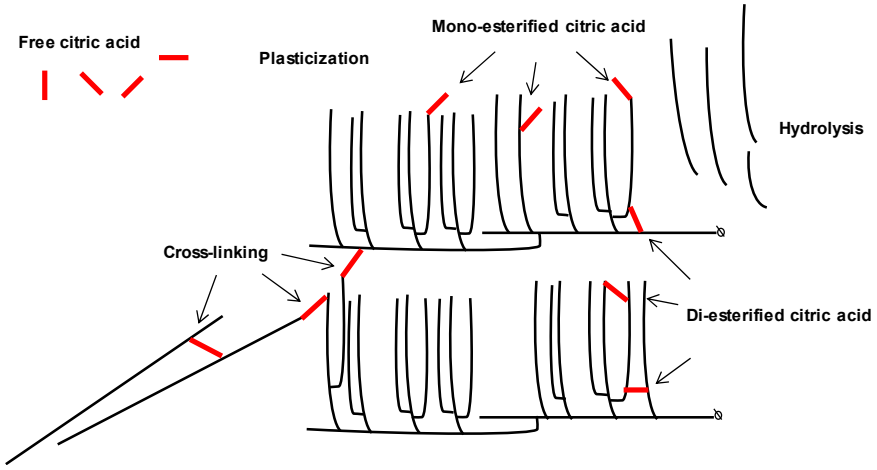


Figure 3. Schematic illustration of possible reactions between starch and citric acid: esterification (mono- and di-ester), cross-linking, hydrolysis and plasticisation.

However, for acid hydrolysis to take place, low pH and high temperature are both needed, as demonstrated by Hirashima *et al.* (2005). Tests by those authors with acid addition before and after gelatinisation showed that no hydrolysis occurred without sufficient temperature (Hirashima *et al.*, 2005; Hirashima *et al.*, 2004). Pre-drying at low temperature to remove water and then raising the temperature to initiate cross-linking can reduce excessive hydrolysis of starch (Wing, 1996).

1.3.3 Genetic modifications

Since the physical properties, and hence the potential applications of starches, are determined by their granule shape and size, amylose/amylopectin ratio and chain length distribution, extensive screening and breeding programmes have

been carried out on different crop varieties. In the past decade, biotechnological tools have helped to produce tailored starches with enhanced functionality, which in fact increases the productivity.

Such recent efforts have produced waxy starches of potato or sweet potato with improved paste clarity and stability (Noda *et al.*, 2002; Visser *et al.*, 1991). In addition, amylose-only starches have been produced in transgenic barley (Carciofi *et al.*, 2012). High-amylose starches are well known to have improved material properties (Jansson & Järnström, 2005; Richardson *et al.*, 2004; Rindlav-Westling *et al.*, 1998; Hermansson & Svegmark, 1996).

1.4 Paper coating in industry and research

In industry, pilot-scale coaters are used to study new coating dispersions. Since such trials are time-consuming and expensive, laboratory-scale experiments mimicking process parameters are used to study the resulting properties of the coating. However, paper coating in industry involves machinery that may differ from the equipment used for laboratory-scale coating and hence the final product might differ (Figure 4). The preparation of the starch formulation can be affected by the use of different chemicals in industry and the laboratory, *e.g*. in terms of the purity of the chemicals, water hardness and ionic strength.

In industry, starch is gelatinised using steam boilers with direct steam, whereas at laboratory scale starch is usually heated in a boiling water bath. The time and shear to reach gelatinisation differ, as different sizes of beakers and stirrers are used and water is added in steam boilers but evaporates in the laboratory beaker. These differences in gelatinisation may cause differing thermal degradation of starch.

Another factor influencing the coating process is the application and metering technique used for application of the coating. At laboratory scale, the starch formulation is applied by a wire-wound bar at low speed, whereas at industrial scale roll applicators with speeds up to 100-fold higher are used. The different shear forces can cause coat weight variations, compression of the base paper and differences in orientation and particle aggregation.

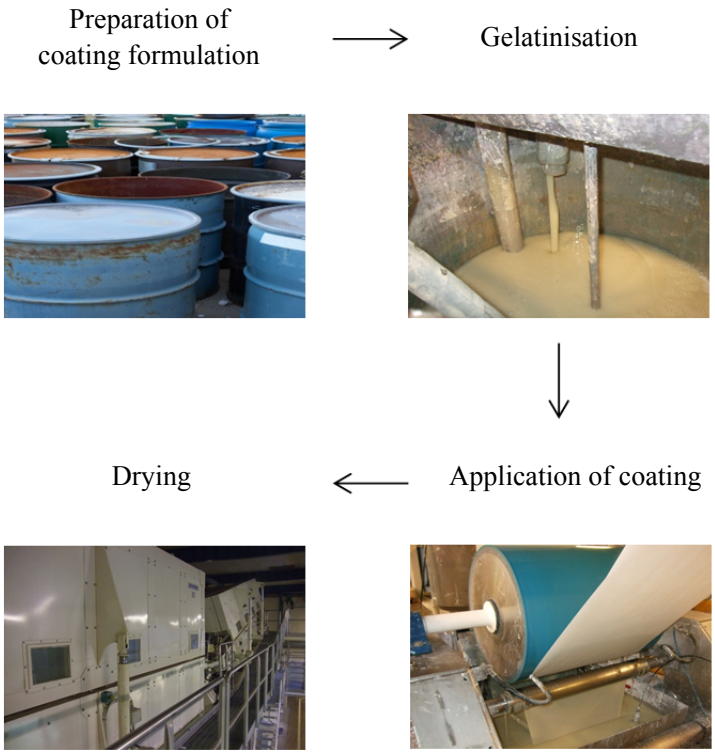


Figure 4. Schematic diagram of the coating process in industry. (Photo source: Carolin Menzel)

The largest differences between pilot-scale and laboratory-scale coatings probably arise during the drying process. In industry, the coated paper runs through infrared dryers with high-energy output for a short time (4 s). Using bench coaters in the laboratory, papers are often dried for about 90 s at lower temperatures. This can result in changes in the film surface but also in molecular structure, *e.g.* degradation of the starch.

2 Aims of this thesis

The overall aim of this thesis was to study the relationship between the molecular structure of starch and material properties in coating materials for food packaging. This was achieved by the following specific aims:

- To develop suitable methods for the detection of cross-linkages in starch by citric acid and to characterise solution-cast films produced by different process conditions (**Paper I**)

- To use citric acid as a cross-linker for starch in order to reduce water sensitivity (**Papers I-II**)

- To identify the relationship between molecular structure and barrier properties of starch coatings containing citric acid (**Paper II**)

- To study different processing effects, *i.e.* curing and pH, to alter starch structure, such as cross-linking and hydrolysis using citric acid (**Papers I-III**), to improve film formation and reduce gas permeation

- To compare the impact of the coating process at laboratory and pilot trial scale on molecular changes in starch coatings (**Paper III**)

- To identify the starch structure of high-amylose potato lines and relate it to their film-forming and barrier properties (**Paper IV**)

3 Materials and methods

3.1 Starches

In Papers I-III, two commercially available, chemically modified potato starch (trade name Solcoat P55 and Solcoat 155; kindly provided by Solam, Kristianstad, Sweden) were used to produce citric acid cross-linked films and coatings. The two starches were hydroxypropylated and oxidised. The difference between the two starches was due to a higher degree of oxidation in Solcoat P55, featuring lower viscosity for industrial applications and a higher solid content while boiling (30% for Solcoat P55 compared with 20% for Solcoat 155).

In Paper IV, starches from high-amylose potato lines with an altered chain length distribution were characterised and used to study the film formation properties of starches exhibiting higher amylose content. These potatoes were grown in the greenhouse and starch was extracted according to Larsson *et al.* (1996).

3.2 Film and coating preparation

Solution-cast films (Paper I) were prepared using a 10% (w/w) starch (Solcoat 155) solution stirred for 45 min in a boiling water bath (Figure 5).

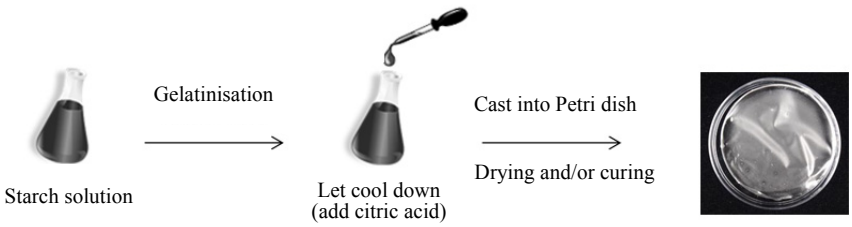


Figure 5. Schematic illustration of preparation of solution cast films with citric acid.

After the solution had cooled down to room temperature, different amounts of citric acid were added (5, 10, 20 or 30 parts per 100 (ppH) parts of dry starch) and the solutions were immediately cast into Petri dishes (8.8 cm diameter) and dried at 70 °C for 5 h in an oven. Curing was carried out on pre-dried films for 10 min at either 105 °C or 150 °C. Starch films with adjusted pH (paper II) were treated similarly, but after addition of 30 pph citric acid a 10 M NaOH solution was used to adjust the pH of the starch solution to 3, 4, 5 or 6.5.

Solution casting of potato starches with a high amylose content (Paper IV) was performed using 3% (w/w) starch solutions that were heated to 140 °C for 45 min under permanent stirring. The solutions were then allowed to cool down to <100 °C and 4.2 mL were transferred to a Petri dish (8.5 cm diameter) and the solvent was evaporated at 23 °C overnight.

For laboratory-scale starch coatings (Papers II-III), a bench coater (K202 Control Coater, RK Coat Instrument Ltd., Royston, UK) with a wire-wound bar was used to apply the starch solutions in double layers on Super Perga WS Parchment 70 g/m^2 paper (Nordic Paper, Norway). Each coating was allowed to dry at 70, 105 or 150 °C for 90 s before a second layer was applied.

Pilot-scale coating (Paper III) was performed at UMV Coating Systems AB, Säffle, on the same paper as above. The same starch formulation with 30 pph citric acid at pH 4, but a slightly different starch (Solcoat P55), was used. The starch formulation also contained 0.01% (w/w) defoamer BIM 7640 and two industrial fillers, 3 pph nano-sized clay filler and 87 pph kaolin filler, to improve barrier properties. Coatings were applied in single and double layers using a hard tip technique at 400 m/s and dried with infrared dryers at 150 °C, followed by 60 °C at 35% relative humidity.

3.3 Methods

3.3.1 Molecular size distribution and molecular weight

In all papers (I-IV), differences and changes in amylose and amylopectin distribution were determined using gel permeation chromatography on a Sepharose CL-2B column (1.6 cm x 90 cm; GE Healthcare, Uppsala, Sweden), with 0.01 M NaOH as eluent at a flow rate of 0.4 mL/min, and fractions of 1 mL were collected. The distribution of amylose and amylopectin was detected using phenol-sulphuric acid reagent (DuBois *et al.*, 1956) and iodine staining (Morrison & Laignelet, 1983).

Molecular weight was determined after size separation on high-performance size-exclusion chromatography columns (HPSEC) connected to a multi-angle

laser-light scattering (MALLS) detector and refractive index (RI) detector, as described elsewhere (Andersson *et al.*, 2009).

3.3.2 Amylose content

In Paper IV, the amylose content of wild-type starches and starches from genetically modified potatoes was determined using two different detection methods, colorimetry and gel permeation chromatography. The basis for amylose determination using colorimetry is its property of colour formation depending on the chain length of the starch polymer, and the method used was that developed by Chrastil (1987). However, one drawback of this method is that no difference can be deducted for interference from colour formation of long-chain amylopectin molecules, and hence the technique can give an overestimation of the amylose content. For that reason, gel permeation chromatography after de-branching of starch was used in this thesis to differentiate between long-chain amylose molecules and short-chain molecules from amylopectin. Therefore, starch was debranched using isoamylase (glycogen 6-glucanohydrolase; EC 3.2.1.68; from *Pseudomonas* sp.; specific activity 280 U/mg) and pullulanase (amylopectin 6-glucanohydrolase; EC 3.2.1.41; from *Klebsiella planticola*; specific activity 42 U/mg) obtained from Megazyme (Wicklow, Ireland), in a 0.1 M NaOAc pH 5.5 buffer (Bertoft & Spoof, 1989). Separation was performed using a Sepharose CL-6B column (90 cm x 1.0 cm) with 0.5 M NaOH as eluent at a flow rate of 0.5 mL/min. Fractions of 0.5 mL were collected and analysed with phenol-sulphuric acid reagent (DuBois *et al.*, 1956). A potato amylopectin standard (Lyckeby Stärkelsen, Sweden) was used to indicate the limit between amylose and amylopectin. Amylose content was determined as area under the curve eluting until 85 mL.

3.3.3 Chain length size distribution

Size distribution of debranched samples was determined using gel permeation chromatography on a Sepharose CL-6B column as described above. Furthermore, high-performance anion-exchange chromatography coupled with a pulsed amperometric detector was used to identify the exact chain length distribution (Koch *et al.*, 1998). Thereby, each number of chain length could be quantified.

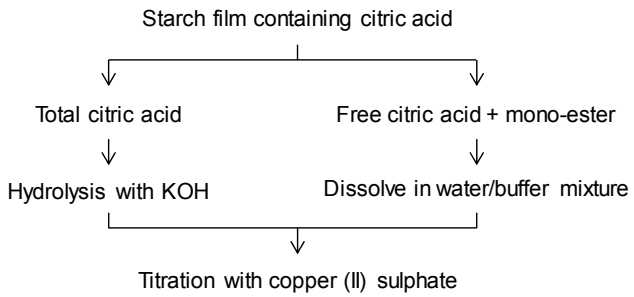
3.3.4 Detection of citric acid di-ester by copper titration

Citric acid can be quantified using complexometric titration with copper(II) sulphate (Klaushofer *et al.*, 1979; Graffmann *et al.*, 1974). It has been shown that besides free citric acid molecules, asymmetric citric acid mono-methyl

esters form a similar 1:1 complex with copper(II) ions (Klaushofer & Bleier, 1983). Hence, two titrations were performed to detect free and mono-esterified citric acid in the intact film and the total amount of citric acid after hydrolysis with KOH (Figure 6). Starch films or starch-coated papers were cut into pieces and either swelled with 2 mL water for about 20 min or hydrolysed with 50 mL 0.1 M KOH for 20 min in a boiling water bath to break all citric acid ester bonds. Afterwards, the pH of the solution was adjusted to 8.5 with 5 N acetic acid and 25 mL 0.1 M borax/boric acid buffer (pH 8.5) and the volume was made up to 250 mL with water. Murexide (Merck, Darmstadt, Germany) was used as the indicator of the end-point of the titration. Both solutions, hydrolysed and non-hydrolysed, were titrated with 0.02 M copper (II) sulphate solution. The amount of citric acid was calculated by assuming that 1 mL of consumed solution was equivalent to 3.842 mg citric acid. The amount of citric acid di-esters was calculated as the difference between the titration for the hydrolysed and non-hydrolysed starch films. Furthermore, the degree of di-esterification (DDE) was calculated as:

$$DDE = \frac{2 * m_{CA} * w_{diester} * M_{AGU}}{M_{CA} * m_{starch}} \qquad \text{(equation 1)}$$

where m_{CA} is the amount of citric acid added (5, 10, 20, or 30 g), $w_{diester}$ is the weight fraction of citric acid taking part in a diester linkage, given as a percentage, 2 is a factor to reflect that two anhydroglucose units are esterified by one citric acid molecule, M_{AGU} is the molar mass of one anhydroglucose unit (162 g/mol), M_{CA} is the molar mass of citric acid (192 g/mol), and m_{starch} is the amount of starch in the film (100 g).



Calculation of citric acid di-ester = Total citric acid – (free citric acid + monoester)

Figure 6. Schematic illustration of citric acid di-ester determination using copper titration.

3.3.5 Confirmation of cross-linking by changes in molecular weight

Molecular weight determinations before and after de-esterification were used to confirm cross-linking of starch by citric acid. Samples were suspended only in water to keep citric acid ester linkages intact and weight-average molecular weight (M_W) before and after de-esterification with NaOH were measured on the water-soluble part of the starch films. About 25 mg starch or 100 mg of the coating containing starch, cut into pieces, were suspended in 5 mL water at 70 °C for 2 h. An aliquot was filtered through a 0.45-µm filter and injected into the HPSEC-MALLS-RI system. Another aliquot was de-esterified with 0.1 M NaOH for 2 h at room temperature before M_W determination.

3.3.6 Water solubility

Water solubility of solution-cast films and starch coatings (Paper I-III) was measured using phenol-sulphuric acid reagent (DuBois *et al.*, 1956). For this, a starch/water suspension of about 5 mg/mL was stirred for 2 days at room temperature. An aliquot was diluted (1:80, v/v) and filtered through a 0.45-µm filter before analysis. Soluble starch content was calculated as glucose concentration using a glucose calibration curve and corrected by a factor of 0.9, based on anhydroglucose units (M=162 g/mol) as the main subunits of starch.

3.3.7 Physical properties

Pasting properties of the starches in Paper IV were analysed using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia) and method *std1* as defined by the manufacturer with a total run time of 13 min.

Barrier properties of films and coatings were determined in Papers II-IV. Water vapour transmission rate was measured according to ISO2528 using silica gel as desiccant. In Papers II-III, oxygen transmission rate was determined according to ASTM D3985-05 by a Mocon® OxTran® oxygen transmission rate tester (Mocon Inc., Minneapolis, MA, USA). In Paper IV, oxygen transmission rate was determined according to ASTM F1927-07.

Thermal analysis was performed by modulated differential scanning calorimetry on a DSC Q2000 system (TA Instruments, New Castle, USA).

Tensile test on starch films was performed according to ASTM D882-02. Before punching out dumbbell shaped specimens, the samples were kept in an air-conditioned climate chamber at 23 °C and 50% relative humidity.

3.3.8 Microstructural studies of starch granules, starch films and coatings

A light microscope (Nikon Eclipse Ni-U microscope, Tokyo, Japan) was used to identify the size and shape of starch granules in high-amylose potato lines (Paper IV). Starch samples were coloured with iodine solution or directly observed with polarised light to detect crystallinity, seen as Maltese crosses.

Solution-cast starch films from Paper IV were embedded in TAAB resin and semi-thin sections (1.5 µm) were cut, stained with 15 µL 0.01% iodine solution and observed with light microscopy. Thin sections (75 nm) were collected on gold grids, stained according to the periodic acid-thiosemicarbazide-silver proteinate method described by Thiéry (1967) and examined with a Philips CM/12 transmission electron microscope (TEM) at an acceleration voltage of 80 kV.

In Paper III, the film surfaces of laboratory and pilot-scale starch coatings were studied with scanning electron microscopy (SEM) using an environmental tabletop Hitachi TM-1000-mu-DeX instrument and an accelerating voltage of 15 kV, magnification x100.

4 Results and discussion

The aim of this thesis was to characterise and relate different starch structures to material properties. In Papers I-III, starch was chemically cross-linked with citric acid, whereas in Paper IV high amylose content was expected to improve film and barrier properties. Three hypotheses were proposed:

> **I.** Citric acid acts as a cross-linking agent between starch molecules and increases molecular weight. Cross-linking in the starch material enhances water resistance and decreases gas permeation.
>
> **II.** A concurrent reaction to cross-linking by citric acid is hydrolysis, where citric acid reacts as acid and degrades starch molecules to smaller molecules, influencing film formation and increasing brittleness.
>
> **III.** Linear chains of amylose form more entanglements and hydrogen bonds, which increases the strength and flexibility of films. A closely-packed structure enhances gas barrier properties.

First, the molecular changes in starch due to hydrolysis and cross-linking were examined on starch films considering different citric acid concentrations and drying temperatures to induce cross-linking of starch. In a second step, citric acid starch formulations were adjusted to industrial conditions by increasing the pH to prevent starch degradation and corrosion of equipment and facilitate easier handling in industry. Barrier properties were related and explained by structural changes in starch. In addition, the effect of the coating process on starch structure and properties was evaluated using three models: free-standing films (solution-casting), laboratory-scale coating (bench coater) and industrial-scale coating (pilot plant). A different approach was used in Paper IV, where

the molecular structure of starches from high-amylose potato lines was related to film formation and barrier properties.

4.1 Molecular changes of starch – degradation due to hydrolysis

In Papers I-III, citric acid was used as a cross-linking agent and possible plasticiser to improve material properties of starch films and coatings. However, a concurrent reaction to cross-linking was hydrolysis, resulting in degradation of starch molecules, which was expected to affect material properties. Hence, molecular changes in amylopectin and amylose due to acid hydrolysis were examined using gel permeation chromatography and molecular weight measurements. Three parameters were studied: citric acid concentration (5 to 30 pph), drying temperature (70 °C, curing at 105 °C or 150 °C) and starch solutions adjusted to higher pH (3 to 6.5). Results from the gel permeation separation are shown in Figure 7. There were two main peaks visible; a rather sharp peak at the beginning of the chromatogram followed by a second broad peak. Large amylopectin molecules (lines in Figure 7 represent glucose concentration) eluted first and had a typical maximum absorbance of the iodine complex (λ_{max}) of 540-550 nm (dots in Figure 7). Amylose molecules eluted later in the chromatogram and had typical λ_{max} at 620-630 nm (Altskär *et al.*, 2008; Morrison & Laignelet, 1983). However, for degraded starch this method did not differentiate between amylose molecules and partially degraded amylopectin molecules or degraded amylose molecules eluting at about the same time and only a lowering of λ_{max} indicated co-elution.

As expected, increasing citric acid concentration progressively degraded starch molecules to smaller molecules, seen as a change in the profile in the chromatogram (Paper I, lines in Figure 1a). Starch films cured at 150 °C with 30 pph citric acid exhibited the strongest degradation of starch due to acid hydrolysis and only one peak was visible in the chromatogram, corresponding to small amylopectin and amylose molecules, whereas films cured at 105 °C showed similar profiles to non-cured films. One reason could be that at high temperature, 150 °C, residual moisture in the film evaporated, in turn increasing the effective acid concentration and thus enhancing hydrolysis. Similar results of high temperature promoting hydrolysis have been reported by Shi *et al.* (2007).

The treatment at 30 pph citric acid resulted in a pH of 2 in the starch formulation and this low pH can cause corrosion issues in industrial applications. Hence, in order to accommodate industrial needs and prevent degradation of starch polymers, pH adjustments were made to the starch solutions treated with 30 pph CA (Paper II). It was found that already at pH ≥4,

hydrolysis stopped for non-cured films and films cured at 105 °C, while at pH ≥ 5 it was possible to subject films to high temperature curing without any notable degradation of starch (Figure 2 in Paper II). Similar pH adjustment between 4 and 6.5 has been suggested previously to prevent starch degradation in production of starch citrates (Klaushofer *et al.*, 1978).

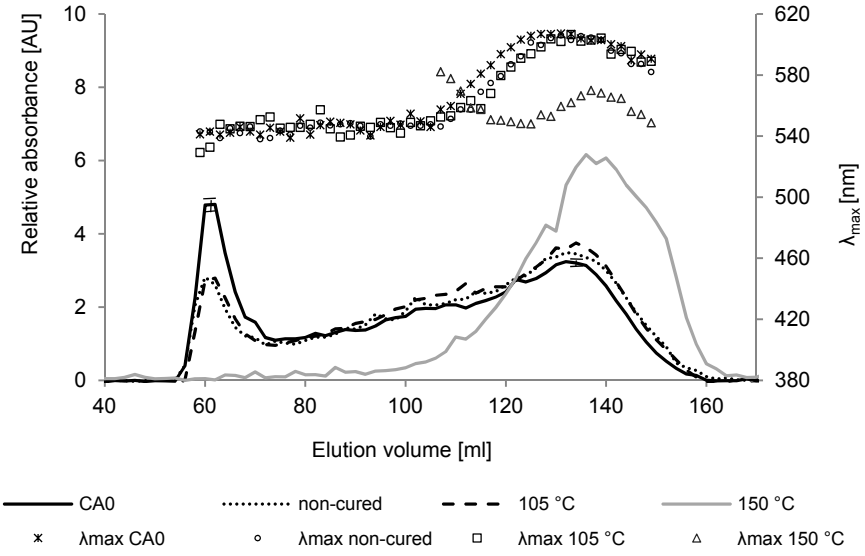


Figure 7. Chromatogram (lines represent glucose concentration of eluting molecules) and λ_{max} (dots represent wavelength at maximum absorbance of iodine complex) of starch films without citric acid, CA0, non-cured, and with 30 pph citric acid non-cured and cured (105 °C and 150 °C), fractionated on Sepharose CL-2B (error bars are indicated at 62 and 134 mL elution volume for CA0). (Source: Menzel *et al.* (2013) with permission from Elsevier.)

Another method used to detect degradation of starch was measurement of M_W using MALLS coupled with RI detection. For comparison, starch films without citric acid had a M_W of about 9.0 x 10^6 g/mol to 9.6 x 10^6 g/mol for non-cured and cured films. The lowest M_W (0.2 x 10^6 g/mol) was observed in films with 30 pph citric acid cured at the highest temperature and, in general, increasing citric acid content resulted in lower M_W. The effect of curing temperature was more pronounced for 150 °C, whereas films cured at 105 °C showed similar results to non-cured films. The results from determination of the distribution of amylose and amylopectin described above (measured as sum of the glucose concentration eluting between 55 and 70 mL) was strongly correlated with molecular weight measurements, as seen in Figure 8. Molecular weight values for starch films with adjusted pH (Table 1 in Paper II) were in agreement with

the amylopectin and amylose distribution results. There was no significant change in M_W compared with the starch films without citric acid when starch films were prepared at pH 4 and cured at 105 °C or prepared at pH 5 and cured at 150 °C.

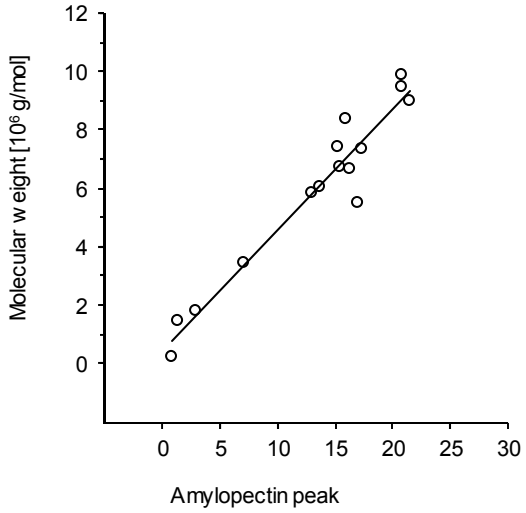


Figure 8. Scatterplot of molecular weight and amylopectin peak (peak area under 55-70 mL) of starch films with and without citric acid, cured and non-cured (R^2= 0.935). (Source: Menzel *et al.* (2013) with permission from Elsevier.)

4.2 Identification and quantification of cross-linking of starch

Cross-linking of starch with citric acid was intended to enhance water resistance and reduce gas permeability. Commonly used techniques to measure cross-linking are swelling experiments, *e.g.* ASTM D2765-11. However, the aim in this thesis was to find a method for measuring the amount of citric acid that is di-esterified and to differentiate between intra- and intermolecular cross-linkages.

Since citric acid is a tri-carboxylic acid, it can react with hydroxyl groups of starch to form esters and can form cross-linkages between starch polymers. In order to detect these ester bonds, a method to detect di-ester of citric acid was devised in Paper I. The method is based on the complex formation between free citric acid and asymmetric citric acid mono-esters with copper (II) ions, as described by Klaushofer and others (Klaushofer & Bleier, 1983; Klaushofer *et al.*, 1979; Graffmann *et al.*, 1974).

The difference between the titration of citric acid (Figure 6) before hydrolysis (free and mono-esterified) and after hydrolysis (total citric acid) allowed the quantitative calculation of citric acid di-esters. The degree of di-esterification indicates cross-linking of starch by citric acid. However, the method does not indicate if these di-esters are intermolecular between two starch polymers or intramolecular within the same starch polymer (Bleier & Klaushofer, 1983).

In Paper I, solution-cast films with four different citric acid concentrations (5, 10, 20 and 30 pph) were analysed. In all citric acid-containing starch films, di-esters were detectable, representing 2.2-21.3% of total added citric acid. Starch films with 30 pph citric acid exhibited the highest DDE range of 0.008-0.054 (Figure 10). Curing of starch films at high temperature was not necessary to initiate di-ester formation and did not increase the amount of di-esters for films with 5 to 20 pph citric acid. This is in contrast with findings in previous studies, where high temperature was needed to induce the cross-linking reaction (Reddy & Yang, 2010; Yang *et al.*, 1997; Wing, 1996; Yang & Wang, 1996; Klaushofer *et al.*, 1978).

In addition, molecular weight measurements were carried out on the water-soluble part of the starch films to differentiate between intra- and intermolecular di-esters, *i.e.* a reduction in molecular weight after de-esterification with NaOH would prove intermolecular cross-linking of starch polymer by citric acid. There was a 10 to 85% decrease in molecular weight after NaOH treatment in all starch films, with the largest decrease in films containing 20 and 30 pph citric acid cured at 150 °C (Table 2 in Paper II). As films with 30 pph citric acid also showed the highest di-ester content, they were expected to exhibit the highest cross-linking density. As shown below (see section 4.3), the cured starch films with the highest citric acid amount were highly degraded, resulting in smaller molecules and short chains, which have been proposed to be more favourable to cross-linking reactions due to more flexibility in space (Wing, 1996).

Furthermore, it was shown that cross-linking occurred already at temperatures as low as 70 °C and no high temperature curing is needed. That is crucial for industrial paper coating applications, facilitating process parameters where no high temperature curing will be needed any longer.

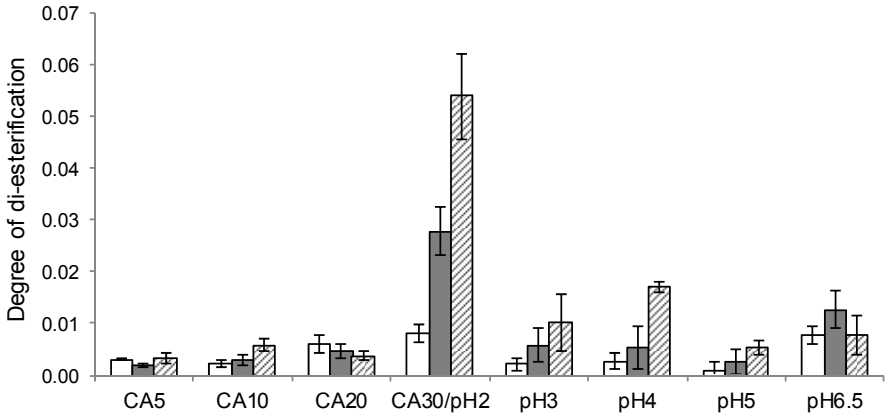


Figure 9. Degree of di-esterification (DDE according to equation (1) of non-cured (white) and cured (105 °C = grey, 150 °C = cross-hatched) citric acid (CA)-containing starch films (number corresponds to CA content in pph) and films with 30 pph citric acid and adjusted pH (error bars represent standard deviation of triplicate samples).

As previously mentioned, in Paper II the same starch formulation with 30 pph citric acid was chosen to improve barrier properties, but pH was adjusted from 2 up to 6.5 to reduce the degradation of starch due to high acid content and to adjust to industrial conditions. As seen in Figure 10, even at pH values as high as 6.5, di-esters were detectable and corresponded to up to 5% of total added citric acid. However, an increase from pH 2 to higher values resulted in lower di-ester content in all films, which can be explained by the reaction mechanism for the ester bond formation (Figure 2), as low pH catalyses the reaction towards the ester product.

At the same time, it was shown that curing increased the di-ester formation for films at pH 2 to 5. No such trend was seen for starch films at pH 6.5, which could be due to the precision of the method. A reason for higher di-ester formation could be that high temperature can reduce the residual moisture due to evaporation during curing at 105 or 150 °C, which in turn affects the reaction equilibrium towards higher yield of ester bonds.

Molecular weight measurements of the water-soluble part of the starch films before and after NaOH treatment revealed cross-linking of starch by citric acid in all films at all pH values. The reduction in molecular weight was highest for films at pH ≤ 4 and at high curing temperature (Table 1), which is in accordance with the di-ester content results (Figure 8).

However, one drawback of this method is that only the water-soluble part of the starch film is measured and the water-insoluble part is expected to have even higher cross-linking density.

Table 1. *Weight-average molecular weight (M_W) of the water soluble part of starch films before (water) and after de-esterification (+NaOH) and decrease in M_W after de-esterification of cured and non-cured starch films produced at different pH values of starch-containing solution. Error limits indicate standard deviation based on duplicates values*

Curing	M_W [10^6 g/ mol] and decrease in M_W [%] after de-esterification				
	pH 2	pH 3	pH 4	pH 5	pH 6.5
Non-cured					
Water	0.41±0.11	8.1±1.81	9.5±0.07	8.8±0.54	10.3±0.09
+ NaOH	0.33±0.01	5.1±1.26	8.7±0.22	8.5±0.63	10.1±0.66
decrease	19%	37%	8%	3%	2%
105 °C					
Water	0.27±0.01	8.3±1.15	9.6±0.38	8.5±1.10	10.8±0.04
+ NaOH	0.22±0.02	4.9±0.70	8.5±0.01	8.1±0.81	10.1±0.06
Decrease	18%	41%	11%	4%	6%
150 °C					
Water	0.34±0.01	0.16±0.01	0.19±0.01	7.8±0.95	10.2±0.60
+ NaOH	0.051±0.03	0.13±0.01	0.15±0.06	6.5±0.55	9.5±0.51
Decrease	85%	19%	21%	18%	7%

4.3 Effect of citric acid on barrier properties of starch coatings

This thesis endeavoured to relate starch structures to material properties in order to produce and predict properties fulfilling industrial demands. Cross-linking was expected to reduce the water sensitivity and gas permeability of starch-based materials. Barrier properties of the same starch films described in this thesis (Paper I) containing 5, 10, 20 or 30 pph citric acid are described in more detail in a previous publication (Olsson *et al.*, 2013). In that study, it was shown that addition of citric acid reduced moisture content and diffusion coefficient up to higher relative humidity values than starch films containing glycerol as plasticizer. Olsson *et al.* (2013) also found that curing had an impact on diffusion coefficient and moisture sorption. Curing at 150 °C reduced diffusion at high relative humidity and resulted in decreased water vapour permeability for starch films with 20 pph and 30 pph citric acid. Olsson *et al.* (2013) suggested that this is mainly attributable to the cross-linking reaction[1] influencing swelling behaviour and molecular movement. As shown above, starch films with 30 pph citric acid exhibited the highest cross-linking

1. The increase in cross-linking for films containing 20pph citric acid could not been shown in di-ester content shown in Figure 10.

content. The chemical bridges between the starch polymers resulted in a more rigid structure of the material that became less susceptible to swelling in liquid water or high relative humidity. Reducing the moisture content and minimising the molecular movement reduced the diffusion of small molecules through the films.

In addition, moisture content results revealed an even more complex behaviour, suggesting phase separation for non-cured and 105 °C cured films with low citric acid content and also high citric acid content. Glass transition temperature results (Paper II) confirmed anti-plasticisation behaviour for films with up to 10 pph citric acid. Furthermore, it was shown that citric acid acted as an internal and external plasticiser. The addition of plasticiser can help to prevent the formation of pinholes, cracks or voids, which are the main cause of molecular transport through a polymer film.

An increase in pH of the starch solution with 30 pph citric acid to 3, 4 and 5 resulted in a reduction in moisture content at 50% relative humidity in cured starch films. This could be explained by declining hydrolysis of the starch material. Increasing curing temperature decreased the moisture content at pH 3, 4 and 5, probably due to increasing degree of di-esterification, whereas at pH 6.5 the low degree of di-esterification resulted in much higher moisture contents.

For all pH-adjusted films, an increase in curing temperature led to a lower water vapour transmission rate (Figure 11), by reducing the starch chain mobility. The reason could be the lower moisture content and the increasing cross-linking of the starch material, at least for pH values between 3 and 5. There was a minimum in water vapour transmission rate at pH 4 at all drying temperatures. This was the point with the lowest moisture content and where cross-linking reaction still occurred, but hydrolysis had nearly stopped and hence minimised movement in the film.

Oxygen transmission rate results were fairly low for all starch coatings with adjusted pH (between 3 and 8 mL/m^2 & 24-h period) and only pH 6.5 coatings were higher in oxygen transmission rate (Table 6 in Paper II). High curing temperature seemed to slightly lower oxygen transmission rate (OTR) values. Cross-linking could be one reason for the decreased permeability, due to the reduced mobility and free volume of the starch material (Byun *et al.*, 2007). The OTR values at 50% relative humidity of starch coatings with a solution pH between 3 and 5 were comparable to those of ethylene vinyl alcohol coatings.

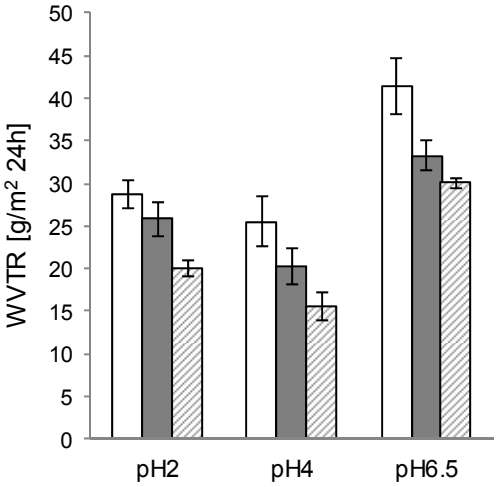


Figure 10. Water vapour transmission rate of starch coatings with 30 pph citric acid (pH 2) and adjusted pH 4 and 6.5, non-cured (white) and cured (105 °C = grey, 150 °C = cross-hatched) (error bars represent standard deviation of triplicate samples).

This shows that it is possible to produce non-toxic barrier coatings based on potato starch and citric acid that are industrially applicable and may provide an alternative to petroleum-based products.

4.4 Impact of the coating process on molecular structure in starch-based barrier coatings cross-linked with citric acid

Testing new starch formulations as barrier coatings in industrial pilot-scale trials is expensive and time-consuming, and hence model systems in small scale are used to identify the potential of new formulations. Solution casting is the most common technique used at laboratory scale to measure changes in molecular structure, while bench-coaters are used to apply coatings on paper to study its barrier properties. However, results from such model systems might not be transferrable to materials in reality. In Paper III, the impact of the coating process on the molecular structure of starch was investigated. For this purpose, solution-cast starch films and coated papers with adjusted pH as described in Paper II were used for comparison between films and coatings.

First, a method to extract the starch from the coating on the carrier material was assessed. It was shown that 0.1M NaOH was sufficient to extract 75-82% of the starch applied as a coating and that the analysis was not influenced by interference with the material.

There were substantial differences in molecular structure between coatings and solution-cast films. Molecular distribution of amylopectin and amylose (Figure 1 in Paper III) and molecular weight results (Table 2) revealed no alteration of starch structure caused by the addition of citric acid at different pH and curing temperature in laboratory-scale coated paper. The reason that no starch degradation was detectable in these coatings as opposed to solution-cast films could be reduced hydrolysis reaction, since there were differences in the drying process between casting and coating. In coating, the heat treatment (70, 105 or 150 °C) was applied for only 90 s after the application of the starch solution on the carrier material, whereas 10 min was used for solution-cast films.

Table 2. *Weight-average molecular weight (M_W) in 0.1M NaOH and water solubility at different pH of laboratory-scale coatings and solution-cast films non-cured and cured at 150 °C*

Sample	M_W [x10^6 g/mol]		Water solubility [%]	
	Non-cured	Cured 150 °C	Non-cured	Cured 150 °C
Solution-cast films				
pH 2	6.1 ± 0.08	0.2 ± 0.01	16 ± 1.5	28 ± 1.0
pH 4	9.2 ± 0.14	5.3 ± 0.53	48 ± 1.4	19 ± 4.7
pH 6.5	9.7 ± 0.42	9.5 ± 0.36	39 ± 2.3	43 ± 0.5
Laboratory-scale coatings				
pH 2	7.5 ± 0.36	6.3 ± 0.91	80 ± 0.4	69 ± 1.3
pH 4	7.9 ± 0.12	7.1 ± 0.26	75 ± 0.2	69 ± 4.7
pH 6.5	7.1 ± 0.08	7.6 ± 0.14	68 ± 2.3	63 ± 7.8

The influence of high temperature on acid hydrolysis of starch has been reported by others (Hirashima *et al.*, 2005; Wing, 1996). However, little is known about the effect of different drying conditions on film formation during drying of starch films and coatings. It has been shown that *e.g.* relative humidity and duration of film formation influence crystallinity and phase separation of components (Rindlav-Westling & Gatenholm, 2003; Rindlav-Westling *et al.*, 1998).

As indicated for the cross-linking reaction, the degree of di-esterification (Table 3) and molecular weight (Table 1 in Paper III) before and after de-esterification were determined. In laboratory-scale coated papers, only up to 3.5% of added citric acid was di-esterified compared with up to 21% in solution-cast films. Molecular weight results confirmed the low cross-linking rate, as M_W decreased by only 6% to 26% after de-esterification with NaOH, compared with up to 85% in solution-cast films. Since ester formation is pronounced at higher temperature, the short drying time of coatings (90 s) weakened the formation of di-esters and cross-linkages, respectively. Water

solubility of coatings was higher (63-80%; Table 2) than that of solution-cast films (16-48%), probably due to the lower degree of cross-linking.

Table 3. *Degree of di-esterification and water solubility of laboratory-scale coated papers prepared at different pH and non-cured (70 °C) or cured (150 °C)*

Sample	Degree of di-esterification		Water solubility [%]	
	Non-cured, 70 °C	Cured at 150 °C	Non-cured, 70 °C	Cured at 150 °C
pH2	0.003	0.007	79.9	69.3
pH4	0.010	0.007	74.8	68.5
pH6.5	0.002	n.d.	67.9	63.1

In addition, pilot-scale coatings of starch solutions with 30 pph citric acid and pH 4 were carried out to study and compare the molecular changes and material properties to solution-cast films and laboratory-scale coatings. Similarly to laboratory-scale coated papers, molecular structure detected by molecular distribution (Figure 1 in Paper III) and molecular weight measurements of pilot-scale coated papers showed no significant starch degradation due to acid hydrolysis. However, citric acid di-ester determination revealed that 10% of added citric acid for single-layer paper and 9% for double-layer paper was di-esterified, which is equivalent to DDE of 0.024 and 0.022, respectively. The decrease in molecular weight after de-esterification with NaOH of >42% clearly indicated cross-linking and was consistent with the higher DDE values. The water solubility of pilot-scale coatings was lower (43% and 67% for single- and double-layer paper, respectively), as in laboratory-scale coatings. Hence, cross-linking rate was higher in pilot-scale coatings than in laboratory-scale coated papers, but lower than in solution-cast films.

One reason for the differences in molecular structure could be the drying process used in industry. After the application of the coating, the paper runs for a very short time through infrared dryers with high energy output at a temperature of 150 °C, followed by drying hoods at 65 °C. During the rapid drying process, water evaporates within seconds, promoting *e.g*. cross-linking and thereby affecting film formation and surface morphology.

As expected, barrier properties were affected by coat weight, morphology and coating application used. Higher coat weight, *i.e*. double layer, increased the barrier to water vapour. Furthermore, pilot-scale coatings showed pinholes (Figure 12) that enhanced the penetration of water vapour through the coating. As laboratory coatings were applied with a wire wound rod at 6 m/min, a smooth and even volumetric addition was achieved, whereas in pilot-scale coatings a coating speed of 400 m/min resulted in coat weight variations.

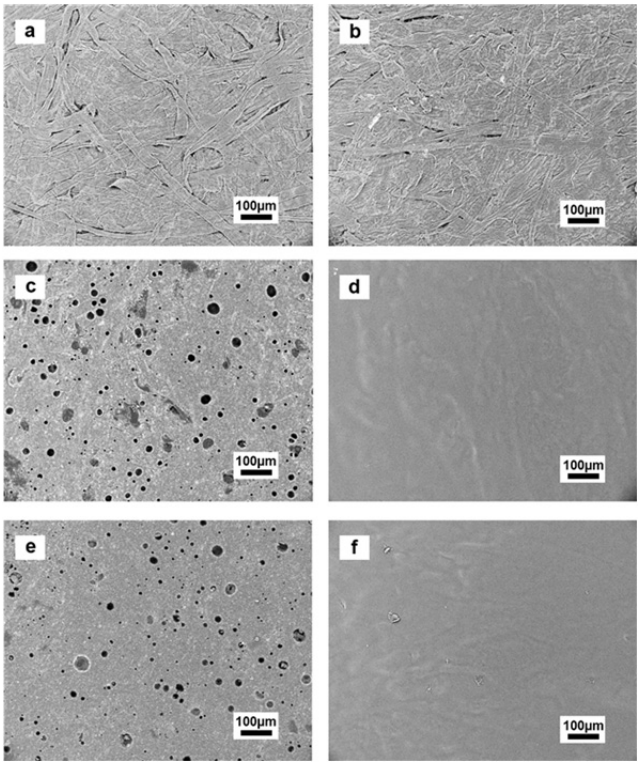


Figure 11. SEM images of: uncoated carrier paper in (a) the pilot-scale trial and (b) the laboratory-scale experiment; coated pilot paper at pH 4 in (c) a single layer and (e) a double layer; and laboratory-scale coatings at pH 4 with (d) no curing and (f) curing at 150 °C. (Source: Menzel and Koch (2014) with permission from Wiley.)

4.5 Effect of altered starch structure on the film formation properties of starch

Besides chemical modification of starch structure to improve starch properties, altered structures and ratio of the two main components within the wild-type plant can influence film formation. Long linear polymer chains can create tight networks and enhance strength and gas barrier of the films (Jansson & Järnström, 2005). Hence, molecular structure of starches from three different potato varieties in native form and after genetic modification by targeted gene suppression by RNA interference was analysed (Paper IV). Each variety showed different amounts of amylose and changed amylopectin structure after genetic modification, providing an estimate of the impact of the structure of granular potato starch on its film formation and barrier properties.

After isolation of starch from potato tubers, amylose content was determined using two methods, colorimetry and gel permeation chromatography (Table 4). Since colour formation with iodine is dependent on the chain length of the starch polymer, the colorimetric technique can give an overestimation of amylose content (Vilaplana *et al.*, 2012). Amylose content of wild-type starches was higher than expected, most probably due to the calibration with isolated amylose and amylopectin standards exhibiting differences in structure, with *e.g.* shorter chains. Hence, gel permeation chromatography was used to obtain better estimates of amylose content and the discussions below are based on those results. The three high-amylose potato lines had an amylose content of 26%, 39% and 49%, as determined by gel permeation chromatography.

Table 4. *Starch content and amylose content of wild-type starches (Kuras, Verba, Dinamo) and their genetically modified types (1068, 7040, 2012) determined colorimetrically using iodine binding and with gel permeation chromatography after debranching*

Sample	Starch content [%][a]	Amylose content [%]	
		colorimetric[b]	Gel permeation[c]
Kuras	86.3 ± 1.35	30 ± 0.3	22 ± 4.0
Modified - 1068	81.5 ± 0.88	45 ± 0.9	26 ± 0.8
Verba	87.9 ± 1.59	31 ± 1.9	23 ± 1.2
Modified - 7040	77.7 ± 2.23	70 ± 2.4	39 ± 0.8
Dinamo	85.3 ± 1.26	30 ± 0.8	23 ± 2.0
Modified - 2012	75.6 ± 2.76	89 ± 1.2	49 ± 5.8

[a] - Starch content (mean±stdev., n=3) determined enzymatically according to Åman *et al.* (1994), based on dry matter, [b] - based on starch content; average and standard deviation (n=4), [c] - average and standard deviation (n=2)

As seen from the gel permeation chromatograms (Figure 13), there were distinct differences between starches, generally not in terms of an amylose content increase, but rather a change in chain length distribution. The elution profile showed that the amount of long chains in amylopectin increased with increasing amylose content, seen as a shift in the chromatogram to lower elution volumes. Amylose molecules could have been altered to exhibit more branching. However, after further studies on separated fractions of amylose and amylopectin, it seemed that amylose was not altered significantly. Similar results have been found by others for starches from genetically modified potatoes with higher amylose content (Hofvander *et al.*, 2004; Blennow *et al.*, 2003; Schwall *et al.*, 2000).

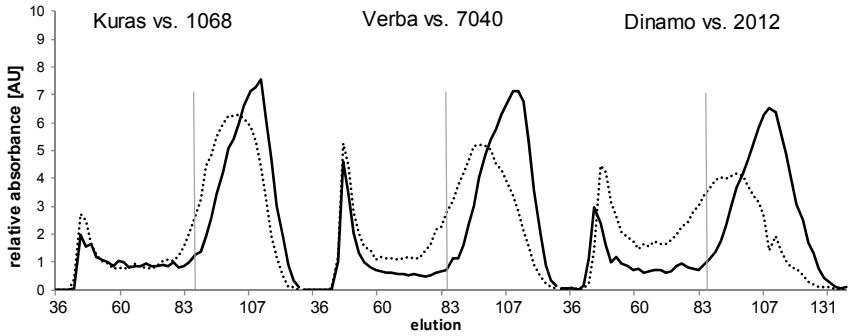


Figure 12. Gel permeation chromatogram on Sepharose CL-6B of wild type potato starches (solid line) and starches of genetically modified potatoes (pointed line) after debranching with isoamylase/ pullanase. Vertical lines represent division of amylose and amylopectin.

High-performance anion-exchange chromatography results for debranched amylopectin revealed increasing chain length with increasing amylose content in modified starches (Figure 14). The average chain length increased from degree of polymerisation (DP) 13 in the wild-type potato starch to DP 15 in 1068, DP 20 in 7040 and DP 22 in 2012. This corresponds to a decrease in short chains in starch 2012 of about 50%. Similar changes have been reported previously (Schwall *et al.*, 2000).

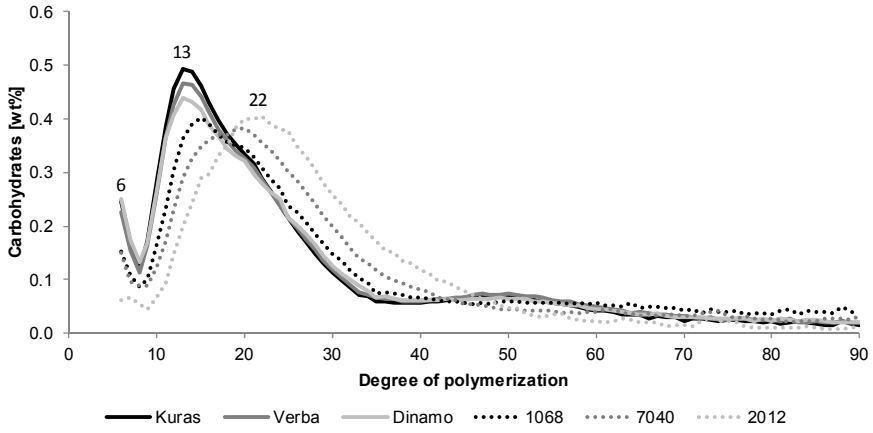


Figure 13. Chain-length distribution after debranching of amylopectin of modified and wild-type starches using HPAEC-PAD.

As a result of the higher amylose content, the shape and size of starch granules were altered. In particular, potato starch granules from lines 7040 and 2012 showed much smaller granules and more irregular shapes such as rod and triangular granules (Figure 15) than wild-type starch with its more spherical

granules. However, the change in amylopectin chain length seemed to have no major effect on crystallinity within the granule, as all granules displayed a Maltese cross under polarised light. In addition, some granules showed overlapping Maltese crosses. According to Jiang *et al.* (2010), amyloplast separation can fail bowing to fusion of two granules by amylose interaction, forming double helices, as shown in high-amylose maize starch. Hence, irregularly shaped granules with a rod or triangular silhouette were created in the potato lines described here.

Pasting properties were also affected by increasing amylose content, resulting in very low or no granule swelling and viscosity during heating for starch lines 7040 and 2012 (Figure 5 in Paper IV). Since it is primarily amylopectin that is responsible for granule swelling, the presence of amylopectin with longer chains and/or higher amylose content alters the pasting properties (Tester & Morrison, 1990).

As a consequence of the low swelling behaviour, higher temperatures were needed to gelatinise the starches 7040 and 2012 and to cast films. Light-microscopy images (data not shown) of longitudinal and cross-sections of films revealed homogeneous structures. Furthermore, scanning electron microscopy images in Figure 15 revealed an uneven surface in films with increasing amylose content which could be remnants of granules remaining in the solution and/or aggregation of amylose molecules during the cooling process (Koch *et al.*, 2010; Bengtsson *et al.*, 2003). It was seen that starches of high-amylose potato lines aggregated very rapidly as immediate cooling of solutions produced gels during casting. Amylose can mainly be expected to be responsible for network formation during cooling of the cast films (Richardson *et al.*, 2004; Rindlav-Westling *et al.*, 1998; Hermansson & Svegmark, 1996; Leloup *et al.*, 1992). This was seen in transmission electron microscopy images of films of starch lines 7040 and 2012, showing a more amylose-like network structure with a more open structure (Figure 15).

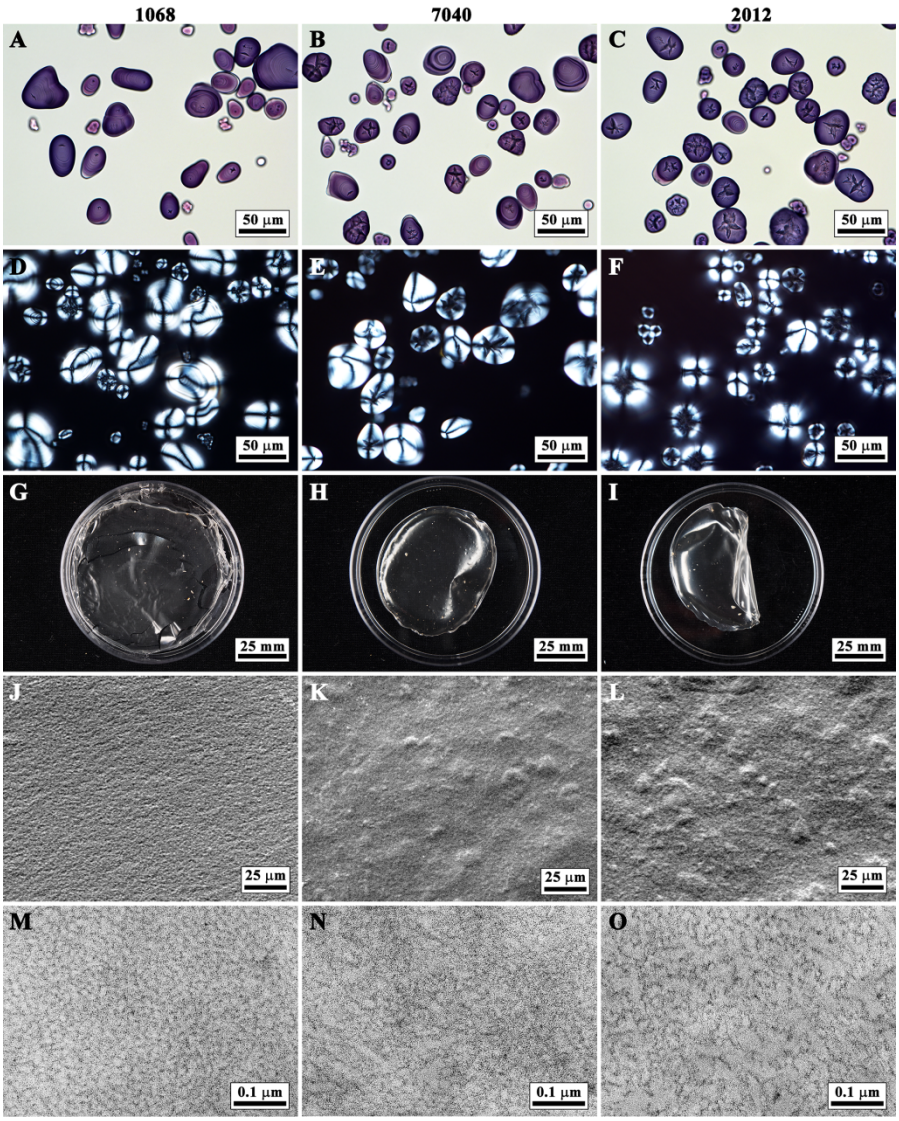


Figure 14. Light microscopy images of starch granules (A to C: stained with iodine, D to F: under polarised light), picture of solution-cast films (G to I), SEM images of film surface (J to L) and TEM images of network structure (M to O) from potato starch lines 1068, 7040 and 2012..

Linear polymers of amylose were expected to form continuous, strong and flexible films. The high-amylose potato lines did not exhibit a significantly higher content of amylose, but rather a changed amylopectin structure, with longer chains influencing film formation and tensile strength.

Stress at break and strain at break increased with increasing amylose content, which is in accordance with previous findings on films with increasing

amylose content (Lourdin *et al.*, 1995). The longer chains in the amylopectin of the high amylose potato lines can increase the interactions between the chains and the formation of double helices. As seen in the transmission electron microscopy, the amylose-like structure is more apparent in potato line 7040 and 2012.

Table 5. *Oxygen transmission rate (OTR) oxygen permeability (OP), stress at break and strain at break of wild-type starch Dinamo and starch lines 1068, 7040 and 2012, high-amylose maize, low-density polyethylene (LDPE) and ethylene vinyl alcohol (EVOH)*

Sample	OTR[a] [cc/m^2 24h]	OP[a] [cc mm/ m^2 24h atm]	Stress at break[b] [MPa]	Strain at break[b] [%]
Dinamo (control)	5.50 ± 0.531	0.170 ± 0.01	341 ± 95.1	1.48 ± 0.28
Modified -1068	4.86 ± 1.850	0.089 ± 0.04	422 ± 36.6	2.79 ± 0.40
Modified -7040	3.87 ± 1.150	0.100 ± 0.03	450 ± 120	2.27 ± 0.81
Modified -2012	2.83 ± 0.415	0.085 ± 0.03	460 ± 124	3.44 ± 2.23
High-amylose maize starch[c]			40	1.92
LDPE[d]		1900	7-16	100-800
EVOH[e]		0.01-12		

[a] – Mean value of duplicates ± standard deviation, [b] – mean value of six replicates ± standard deviation, [c] - from Koch *et al.* (2010), [d]- from Doak (1986), [e] – from Lange and Wyser (2003)

Cast films exhibited a decrease in oxygen transmission rate in all three potato lines 1068, 7040 and 2012 compared with the wild-type potato starch Dinamo (Table 5). All three high-amylose potato lines gave films with lower oxygen permeability compared with the wild-type starch Dinamo. It has been shown by others that amylose constitutes a better oxygen barrier than amylopectin, a finding which they attributed to higher crystallinity in amylose films (Rindlav-Westling *et al.*, 1998).

5 Conclusions

The aim of this work was to improve understanding of the relationship between the molecular structure, material properties and processing of starch dispersions used as coatings in food packaging. Two approaches to creating potential packaging products were studied: reducing the water sensitivity of starch through chemical cross-linking with citric acid and using altered starch structures with longer chains to enhance film formation.

- It was possible to detect cross-linking by citric acid in starch films and coatings using molecular weight measurements and titration with copper (II) ions (**Paper I**).

- It was proven that cross-linking reaction was initiated at temperatures as low as 70 °C and increased with curing temperature at high citric acid content (**Paper I**).

- The two concurrent reactions, hydrolysis and cross-linking, affected the molecular structure of starch and influenced barrier properties (**Papers I, II**). Hydrolysed starch molecules seemed to favour cross-linking reaction, which in turn reduced gas permeation.

- Controlling the pH of the starch solution containing citric acid was shown to reduce hydrolysis and led to reduced gas permeability, with a minimum at pH 4 (**Paper II**).

- Laboratory-scale coatings of starch seem to be a better model system to study and understand molecular changes and their relation to barrier properties than solution cast films (**Paper III**).

- Starches with higher amylose content revealed an altered granule shape and amylopectin structure with longer chains, resulting in stronger films with better oxygen barrier properties (**Paper IV**).

6 Future research

A deeper understanding of the relationship between starch molecular structure and its performance as a film-former and barrier to oxygen and water vapour was achieved by this work. This detailed knowledge about changes within starch will help to predict desired properties to be used in new packaging materials. Future research should:

- Determine the influence of starch structure (lower M_W or pure amylopectin) on cross-linking reaction, as small molecules have more flexibility in space to be cross-linked.

- Study drying kinetics in different techniques such as solution casting and coating applications to improve the translation of pilot-scale parameters to laboratory-scale parameters.

- Test different di- or multi-carboxylic acids for cross-linking reaction, *e.g.* oxalic acid.

- Study the effect of relative humidity, drying time, temperature, ventilation, thickness and solid content in solution on film formation of starch films and coatings.

- Analyse fine structures in terms of clusters and domains of amylopectin structure in high-amylose potato starch and their relation to physical properties.

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