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Molecular structure of citric acid cross-linked starch films

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ABSTRACT

The effect of citric acid (CA) on starch films has been examined. A new method to detect cross-linking of starch by CA in solution-cast films by molecular weight measurements is described. Furthermore, we managed to distinguished between free, mono- and di-esterified CA and quantify di-ester content within starch films by using a modification in the method of complexometric titration with copper(II)-sulfate. Cross-linking of starch by CA occurred at low temperature, 70 °C, which we assumed is so far the lowest temperature reported where cross-linking reaction occurred. This is essential for starch coating applications within paper industry since no high temperatures for curing will be required. However, curing at 150 °C and high CA concentrations, 30 pph, increased cross-linking reaction. Furthermore, the physical properties like water solubility, gel content and glass transition temperature, were highly reflected by changes in the molecular structure i.e. cross-linking and hydrolysis, as well as CA content and curing temperature.

1. Introduction

Starch is a highly promising biopolymer for the production of packaging material since it in native state gives films with good tensile and gas barrier properties and in addition it is renewable, available in high purity at low cost, and fully biodegradable. However, non-plasticized starch films are brittle and their hydrophilic character results in poor moisture barrier properties and high water sensitivity compared with films produced from synthetic polymers such as polyethylene or polypropylene. To improve film formation and material properties of native starch, plasticization and chemical modification, e.g. cross-linking of the starch is required. Plasticizers such as water, glycerol, xylitol or polyethylene glycol reduce the glass transition temperature, T_g, of the polymer matrix and prevent the formation of cracks and pinholes (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002; Godbillot, Dole, Joly, Rogé, & Mathlouthi, 2006; Stading, Rindlav-Westling, & Gatenholm, 2001).

One major goal in recent research is to overcome the hydrophilic and hygroscopic character of thermoplastic starch to improve the barrier properties against both liquid water and water vapor. One approach is to chemically modify the starch, e.g. through cross-linking (Ghosh Dastidar & Netravali, 2012; Olivato, Grossmann, Bilck, & Yamashita, 2012; Reddy & Yang, 2010). A potential and recently discussed cross-linking agent is citric acid (CA) (Reddy & Yang, 2010). Citric acid is a naturally occurring organic acid with three carboxylic groups and is generally classified as a safe food additive. Hence, it can be used in food contact materials. It has been shown that the addition of CA decreases moisture absorption and reduces water vapor permeability in starch films (Ghanbarzadeh, Almasi, & Entezami, 2011; Olsson, Hedenqvist, Johansson, & Järnström, 2013). Studies on the mechanical and thermal properties of starch films have shown that CA improves tensile strength and thermal stability (Ghanbarzadeh et al., 2011; Olivato et al., 2012). In addition to cross-linking, CA also acts as a plasticizer for starch (Ghanbarzadeh et al., 2011; Reddy & Yang, 2010; Shi et al., 2007). Therefore, a CA plasticized and cross-linked starch as water soluble film forming dispersion can offer great potential to be used as barrier coating within the food packaging sector.

Citric acid can react with all three hydroxyl groups in the anhydroglucose monomer and with the hydroxyl group of the reducing end of the chain (Gramera, Heerema, & Parrish, 1966; Klaushofer & Bleier, 1982). Possible structures of esters formed between CA and starch are discussed in detail by Bleier and Klaushofer (1983). An esterification reaction between starch and CA could result in CA mono-, di-, and tri-esters. Di-esters can be formed either intermolecularly between two polymer molecules or intramolecularly

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within the same polymer molecule. An intermolecular cross-link between two different macromolecules obviously increases the molecular weight. For intramolecular di-esterification the formation of a di-ester within the same anhydroglucose monomer, between two neighboring anhydroglucose monomers, or within the same polymer molecule are all sterically possible, but this does not lead to cross-linking. The probability of tri-esters is doubtful (Bleier & Klaushofer, 1983). Klaushofer and Bleier (1983) have shown the occurrence of highly substituted regions and weakly substituted regions in starch. It has been proposed that esterification mainly takes place around the branching points of amylopectin (Klaushofer & Bleier, 1983).

A concurrent reaction to cross-linking in response to CA addition is hydrolysis of starch due to lowering of the pH. In addition, high moisture content increases the hydrolysis rate. It has previously been shown that high temperature, high CA concentration, and low pH increase the degradation rate (Carvalho, Zambon, da Silva Curvelo, & Gandini, 2005; Hirashima, Takahashi, & Nishinari, 2004; Shi et al., 2007; Wing, 1996).

The aim of this study was to find a suitable method for detecting cross-linking of starch by CA and to investigate how CA concentration and curing affects the molecular structure of starch. We aimed to improve our understanding of the interactions between starch and CA in starch-based films cured at different temperatures, in order to facilitate processing of modified starch for industrial production of accessible films or coatings for food packaging and to better understand barrier properties such as diffusion coefficient and moisture content reduction with CA addition shown in a previous study (Olsson et al., 2013).

2. Materials and methods

2.1. Materials

The solution-cast starch films consisted of hydroxylpropylated and oxidized normal potato starch including different concentrations of CA (0, 5, 10, 20 and 30 parts (by wt.) per hundred parts of dry starch, pph) and were produced as described elsewhere (Olsson et al., 2013). In brief, a 10% starch solution was gelatinized in a boiling water bath for 45 min under vigorous stirring. After cooling to room temperature CA was added and the solutions were cast in Petri dishes and dried at 70 °C for 5 h, with or without subsequent curing at either 105 °C or 150 °C for 10 min. All solvents and reagents used were of analytical grade. The pH of the solution-cast starch films was about 6 for solutions without CA and about 2.5 and 1.9 for films containing 5 pph and 30 pph CA, respectively.

2.2. Molecular distribution of amylose and amylopectin

The films were dissolved to a concentration of 5 mg/mL in 0.1 M NaOH at room temperature for 2 h. Aliquots of 1 mL were fractionated by size-exclusion chromatography (SEC), fractions of 1 mL were collected and chromatograms were produced by carbohydrate analysis and iodine staining according to Altskär et al. (2008). The experiment was performed in duplicate.

2.3. MALLS for determination of weight-average molecular weight

2.3.1. M_W after de-esterification

The samples were dissolved as described above and an aliquot of 75 μL was injected into a high-performance size-exclusion chromatography (HPSEC) system connected with multi-angle laser light scattering (MALLS) and refractive index (RI) detector for M_W determination (Andersson, Fransson, Tietjen, & Åman, 2009). The experiment was performed in duplicate.

2.3.2. M_W determination of water-soluble starch content and subsequent de-esterification

The sample preparation in Section 2.3.1 included the dissolution of starch films in 0.1 M NaOH. However, by doing so, ester-linkages formed between CA and starch are hydrolyzed and M_W determination only measures changes in the starch molecules. To detect cross-linking of starch by CA, M_W of the water soluble starch content was measured before and after de-esterification with NaOH. Therefore, film samples (25 mg) were weighed out and 5 mL distilled water was added. The mixture was stirred and heated at 70 °C for 2 h and the water-soluble starch content was filtered through a 0.45 μm filter and an aliquot was directly injected into the HPSEC-MALLS-RI system for M_W determination. Another aliquot was subsequently de-esterified with 0.1 M NaOH for 2 h at room temperature before M_W determination.

2.4. Complexometric titration of CA with copper (II)-sulfate

The principles of the complexometric titration of CA with copper (II)-sulfate is described in more detail elsewhere (Graffmann, Domels, & Sträter, 1974). Klaushofer, Berghofer and Pieber (1979) applied this method to citrate starches to quantify the amount of free CA and to determine the grade of ester substitution (Klaushofer et al., 1979). In brief, copper (II)-ions are able to form a stable complex with CA in a stoichiometric copper (II):CA ratio of 1:1. The endpoint is sharp and can be detected with a metal indicator such as murexide. Klaushofer and Bleier (1983) discovered that asymmetric CA mono-methyl esters form similar complexes and react like free acid. Thus, copper titration will detect free CA, as well as asymmetrically mono-esterified CA.

Using that fact, we modified the method and carried out two independent titrations on the starch films. In one titration, the cross-linkages in starch film samples were hydrolyzed with KOH to measure the total amount of CA, including free and esterified CA. In a second titration, the water-swollen films were titrated directly to measure free plus mono-esterified CA. Hence, this method allowed measuring the difference in titration yield which quantifies the amount of CA di-esters in the starch film that cannot be measured by titration. We believe that this is the first time that di-ester content is quantified.

Starch films of around 300 mg were cut into pieces. These films were either hydrolyzed with 50 mL 0.1 M KOH for 20 min in a boiling water bath or swelled with 2 mL of distilled water for 20 min. After cooling to room temperature, the pH of the solution was adjusted to 8.5 with 5 N acetic acid and 25 mL of a borax/boric acid buffer (pH 8.5). The solution was made up to 250 mL with distilled water and a spatula tip of murexide (Merck, Darmstadt, Germany) was used as indicator. Titration was carried out with 0.02 M copper (II)-sulfate solution where 1 mL consumed copper solution is equivalent to 3.842 mg CA.

The difference in the yield of CA titration between the hydrolyzed and non-hydrolyzed CA measurements was then calculated. The degree of di-esterification, DDE, of starch was calculated as:

$$\mathrm{DDE} = \frac{2 \cdot m_{CA} \cdot w_{diester} \cdot M_{AGU}}{M_{CA} \cdot m_{starch}} \quad (1)$$

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

2.5. *Water solubility*

The amount of water-soluble matter used for M_W determination was determined as anhydroglucose concentration (g anhydroglucose/100 g starch) using the phenol sulfuric acid method described in Altskär et al. (2008) and using a glucose calibration curve multiplying by 0.9 in view of anhydroglucose monomers. The experiment was performed in duplicate.

2.6. *Gel content in formic acid*

The gel content was measured according to Reddy and Yang (2010). In brief, 0.1 g film sample was placed in 5 mL formic acid under mild stirring for both 24 h at 23 °C and 5 h at 50 °C. The gel content was determined as the dry weight of the material that did not pass through a stainless steel mesh (Tyler mesh 30) and was expressed as percentage of dry film weight. The experiment was performed in triplicate.

2.7. *Starch film structure and morphology by wide-angle X-ray scattering (WAXS)*

WAXS measurements were performed at the 911-2 beamline at the MAX-lab Synchrotron, Lund University, Sweden (Mammen et al., 2002). The wavelength was 1.04 Å, and the sample-to-detector distance was 149.8 mm. Silicon powder was used as a calibration standard for peak positions. Two-dimensional images were recorded using an area-CCD detector (active area diameter: 165 mm, Marresearch GmbH, Norderstedt, Germany) with 5 min of data acquisition. Parasitic scattering was subtracted from all diffractograms. Four to five starch film pieces were stacked on top of each other with a total thickness of 1 mm and placed in a sample holder for X-ray scattering. The data were analyzed using the software FIT2D (Hammersley et al., 1995).

2.8. *Thermal analysis*

The effects of CA and curing temperature on T_g of the starch films were determined by modulated differential scanning calorimetry, MDSC. A TA system DSC Q2000 (TA Instruments, New Castle, USA) was used for the experiments. The scans were performed by heating the samples from 0 to 170 °C with an underlying heat rate of 2 K/min, a modulated heat amplitude of 0.318 K, and a period of 60 s. Pre-trials were performed from −70 to 170 °C to detect any glass transition at low temperatures originating from the plasticizer that would prove phase separation. Film samples stored at 23 °C and 50% RH were cut into small pieces weighing 10.05 ± 0.05 mg and placed in hermetically sealed aluminum pans. An empty pan was used as reference. The measurements were performed in at least triplicate. The data were analyzed using TA Universal Analysis 2000 software (TA Instruments, New Castle, USA). The inflection point in the reversible heat flow curve was taken as the glass transition temperature, T_g.

3. Results and discussion

3.1. *Molecular characterization of amylose and amylopectin*

The method was used to identify molecular changes in amylose and amylopectin caused by acid hydrolysis during drying and curing. The chromatographic profiles of the starch films showed two major peaks (Fig. 1a). The first peak eluted between 55 and 70 mL and corresponded to large amylopectin molecules (Altskär et al., 2008). Maximum absorbance, λ_{max}, was about 540–550 nm, which is typical for branched molecules with short α-1,4 linked glucose chains (Morrison & Laignelet, 1983). The second large peak eluting roughly between 110 and 160 mL, and with λ_{max} up to 610 nm, corresponded to amylose, but also to partially degraded amylopectin (Altskär et al., 2008). Results for the amylopectin peaks area (peak area under elution fraction 55–70 mL) are shown in Table 1 and are further discussed below in connection to M_W.

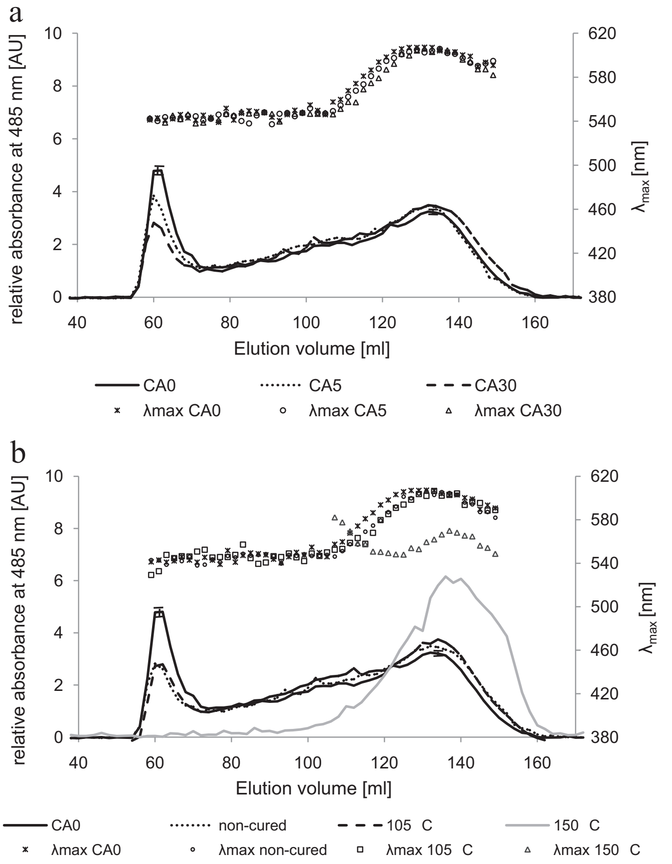

Fig. 1. (a) Chromatograms (lines) and λ_{max} (dots) of starch films CA0, CA5, and CA30, non-cured and fractionated on Sepharose CL-2B (error bars indicated at 62 and 134 mL elution volume for CA0). (b) Chromatograms (lines) and λ_{max} (dots) of the starch films CA0 non-cured and CA30 non-cured and cured (105 °C and 150 °C) fractionated on Sepharose CL-2B (error bars indicated at 62 and 134 mL for CA0).

Films containing CA showed a shift in the chromatogram with increasing CA concentration (Fig. 1a). The relative absorbance of the first eluting peak decreased significantly, whereas it increased concomitantly for the second eluting peak (Fig. 1a). Besides this, λ_{max} for the material eluting between 110 and 160 mL shifted to lower values. Hence, large amylopectin molecules were degraded to smaller size and eluted together with amylose in the second peak, resulting in lower λ_{max} values.

Films cured at 150 °C showed the strongest decrease of the first eluting peak with increasing amount of acid (Fig. 1b). In addition, the chromatographic profile of the starch film containing 30 pph CA consisted of only one peak eluting between 80 and 160 mL (Fig. 1b) indicating the strongest degradation of amylopectin due to acid hydrolysis. Amylose molecules were most likely degraded as well, although the method used did not differentiate between the two molecular fractions. It was shown that all CA-containing

Table 1
Sample abbreviation, amylopectin peak area (peak area under 55–70 mL) and weight-average molecular weight (M_W) of non-cured and cured (105 °C and 150 °C) starch films with and without CA.

Sample abbrev.	CA content [pph]	Amylopectin peak [AU][a]			M_W [10^6 g/mol][a] in NaOH		
		Non-cured	105 °C	150 °C	Non-cured	105 °C	150 °C
CA0	0	21.4 ± 0.46	20.7 ± 1.55	20.8 ± 0.36	9.0 ± 0.37	9.9 ± 0.03	9.5 ± 0.14
CA5	5	17.3 ± 0.34	15.3 ± 0.63	7.1 ± 1.13	7.4 ± 0.27	6.7 ± 0.08	3.4 ± 0.12
CA10	10	16.0 ± 0.52	17.0 ± 0.42	2.9 ± 0.53	8.4 ± 0.26	5.5 ± 0.61	1.8 ± 0.02
CA20	20	16.3 ± 0.94	15.2 ± 0.34	1.3 ± 0.46	6.6 ± 2.13	7.4 ± 0.02	1.5 ± 0.02
CA30	30	13.7 ± 0.28	13.0 ± 1.19	0.74 ± 0.46	6.1 ± 0.08	5.8 ± 0.05	0.2 ± 0.01

[a] Mean value of duplicates ± standard deviation.

Table 2
Weight-average molecular weight (M_W) of water-soluble starch before (water) and after subsequent de-esterification with NaOH (PlusNaOH).

Sample	Water soluble M_W [10^6 g/mol][a]					
	Non-cured		105 °C		150 °C	
	Water	PlusNaOH	Water	PlusNaOH	Water	PlusNaOH
CA0	9.9 ± 0.19	9.6 ± 0.05	9.9 ± 0.20	9.8 ± 0.22	9.2 ± 0.76	8.9 ± 0.78
CA5	8.3 ± 0.99	6.1 ± 0.27	6.5 ± 0.09	5.2 ± 0.42	5.1 ± 0.05	2.2 ± 0.07
CA10	7.4 ± 0.54	5.9 ± 1.05	7.3 ± 0.85	6.2 ± 0.19	6.1 ± 0.05	5.0 ± 0.54
CA20	3.9 ± 0.01	3.3 ± 0.39	4.1 ± 0.43	3.1 ± 0.43	1.8 ± 0.44	0.31 ± 0.44
CA30	0.41 ± 0.11	0.33 ± 0.01	0.27 ± 0.01	0.22 ± 0.02	0.42 ± 0.12	0.13 ± 0.12

[a] Mean value of duplicates ± standard deviation.

films, i.e. non-cured and cured, were significantly degraded compared to starch films without CA (amylopectin peak area, Table 1). However, films with up to 20 pph CA showed only small differences in the chromatographic profile compare to films without CA and hence were only slightly affected by the acid hydrolysis. Starch films cured at 105 °C showed similar profiles to the non-cured films for all CA concentrations. Consequently, curing at 105 °C did not significantly affect starch hydrolysis. We conclude that acid hydrolysis of the glycosidic linkages in starch was favored at high temperature and at high CA concentrations, i.e. 30 pph, which is consistent with previous reports (Carvalho et al., 2005; Olivato et al., 2012; Shi et al., 2007). One reason could be that at high temperature residual moisture could evaporate, increasing the effective CA concentration, thereby lowering pH and hence enhancing hydrolysis of starch. Shi et al. (2007) reported a similar significant hydrolysis of the starch chains when CA content increased above 20% in melt blended starch at elevated temperatures. An adjusted degradation by controlling CA concentration and drying conditions could be used to improve processing properties such as viscosity and open a wide field of applications of CA containing starch films.

3.2. MALLS to determine weight-average molecular weight

The results of the M_W measurements are shown in Table 1. The solution-cast starch films produced without CA had a M_W of about 9.0×10^6 g/mol. In general, M_W decreased slightly with increasing CA content for all cured and non-cured films (Table 1). Films containing 30 pph CA showed the greatest reduction in M_W, from 6.0×10^6 g/mol for non-cured films to 0.2×10^6 g/mol for films cured at 150 °C, implying a M_W decrease by 62–97% compared with the film without CA. The decrease in M_W was due to an induced acid hydrolysis at high temperature as discussed above. There were only small changes in M_W between the non-cured films and films cured at 105 °C, which is consistent with results shown in the amylose and amylopectin distribution (Table 1). The acid hydrolysis of starch evaluated by the determination of amylose and amylopectin distribution (the peak area under the 55–70 mL eluted fraction) were strongly correlated with the measured M_W as seen in Fig. 2 ($r^2 = 0.923$).

3.3. M_W of the water-soluble starch content before and after de-esterification

By comparing the M_W before and after de-esterification with NaOH, cross-linking of starch by CA can be proven. Cross-linking reaction can increase M_W due to covalent ester-bonds of one CA molecule to two starch molecules which, however, can be broken by NaOH resulting in lower M_W. However, only the water-soluble starch content could be studied by this method. The results of the M_W measurements in water are given in Table 2. Starch films without CA were neither degraded by NaOH-treatment nor cross-linked.

The M_W of starch films containing CA showed a 10–85% decrease after NaOH-treatment (Table 2). This evidently proves intermolecular cross-linkages in starch by CA in all starch films, even in the non-cured films. The decrease in M_W was largest for the starch films containing 20 and 30 pph CA cured at 150 °C. In addition, most films containing CA cured at 150 °C had higher M_W in the water-soluble

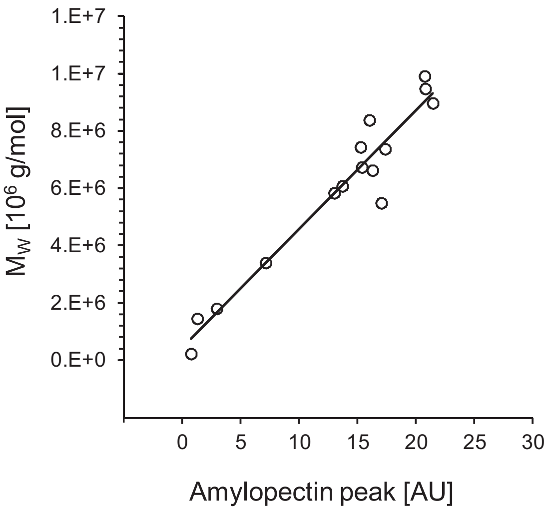

Fig. 2. Scatterplot of molecular weight (M_W) and amylopectin peak (peak area under 55–70 mL) of films with and without CA, cured and non-cured ($R^2 = 0.935$).

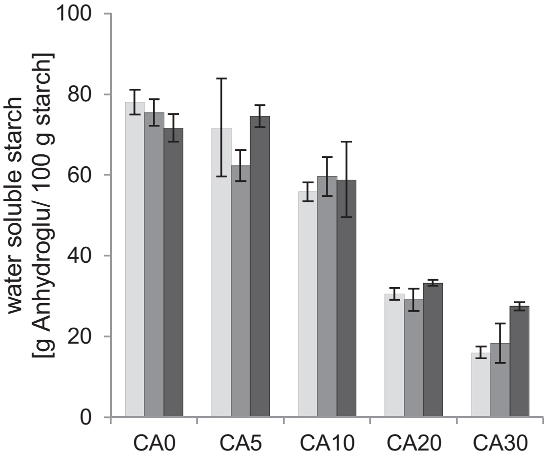

Fig. 3. Water soluble starch content of non-cured (light gray) and cured (105 °C – gray, 150 °C – dark gray) film samples (mean value of duplicates, bars indicate standard deviation).

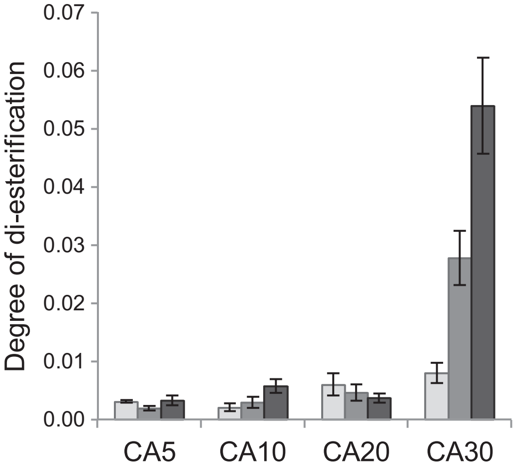

Fig. 4. Degree of di-esterification (DDE according to Eq. (1)) of non-cured (light gray) and cured (105 °C – gray, 150 °C – dark gray) CA-containing starch films (error bars represent standard deviation of triplicates).

starch film part than in 0.1 M NaOH (Tables 1 and 2) which indicates the cross-linking between starch molecules since only cross-linking reaction can increase M_W of starch. Of course, the determination of M_W of the water-soluble starch content is not representative of the whole starch film, since the starch films are only partly soluble in water.

Nevertheless, to the best of our knowledge this is the first time that CA cross-linking of starch has been shown in correlation to M_W change. Further, it was shown that cross-linking occurred already at low temperature drying, i.e. already at drying at 70 °C without a subsequent curing step. This is crucial for industrial applications such as coating of paper where cross-linking has been claimed to be initiated by curing which from these results will not be necessary.

3.4. Water solubility

The results of the water solubility measurements are shown in Fig. 3. Water solubility of the starch films decreased with increasing CA concentration. Cross-linking and esterification reactions can reinforce the starch network both chemically and physically, thus resulting in lower water solubility (Ghanbarzadeh et al., 2011; Krumova, López, Benavente, Mijangos, & Pereña, 2000). Curing did not significantly affect the solubility of starch, which is rather surprising since high temperatures are commonly used to induce cross-linking of starch by CA in order to obtain a more dense structure and to improve resistance to dissolution (Klaushofer, Berghofer, & Steyrer, 1978; Reddy & Yang, 2010; Wing, 1996).

By combining the results from M_W determination after de-esterification, showing low M_W values, and water solubility measurements, suggest that primarily small molecules (both cross-linked and free) were dissolved in water (Table 2 and Fig. 3). Highly cross-linked and high M_W molecules can be expected to remain as water-insoluble matter. Our results showed that the CA concentration had a higher impact on the water solubility than the curing.

3.5. Complexometric titration of CA with copper (II)-sulfate

For the first time, the amount of di-esterified CA was measured as the difference in CA titration for hydrolyzed and non-hydrolyzed starch films and can be expressed as both a percentage of total CA and as DDE of starch (Eq. (1); Fig. 4). In all CA-containing starch films, di-esters were present in concentrations ranging from 2.2 to 21.3% of total added CA (data not shown). The highest concentration of CA, 30 pph, gave the highest DDE, ranging from DDE 0.008 (every 125th anhydroglucose monomer being di-esterified) up to DDE 0.054 (every 19th anhydroglucose monomer being di-esterified) for non-cured and 150 °C cured films, respectively. Citric acid concentrations between 5 and 20 pph resulted in lower DDE values of between 0.002 and 0.006. There was no linear correlation between curing temperature and DDE up to 20 pph CA. These results do not differentiate between inter- or intramolecular di-esters, as described earlier (Bleier & Klaushofer, 1983). However, considering the results from the M_W measurements in water, with M_W decreases of 85% after de-esterification, there is clear proof of intermolecular di-esters, i.e. cross-linkages, in the starch films, since intra-molecular di-esters would not cause a reduction in M_W.

We concluded that the 30 pph CA-containing starch films were highly cross-linked, since they exhibited most CA di-ester, highest M_W decrease after de-esterification and lowest water solubility. Further, the results clearly indicate cross-linkages in starch films without curing at high temperature, which to the very best of our knowledge, have not been achieved or demonstrated by any other study.

3.6. Gel content in formic acid

Measurement of the gel content of a polymer in a selected solvent indicates the amount of a film that is sufficiently cross-linked (Larson, 1999). There was no gel content detectable in films subjected to formic acid for 24 h at 23 °C containing 0, 5 or 10 pph CA indicating that cross-linking reaction was not sufficient. Further, for film with 20 pph CA only the film cured at 150 °C had measurable gel content (23.2%). However, films containing 30 pph CA

Table 3
T_g from MDSC for non-cured and cured (105 °C and 150 °C) starch films with different CA content.

Sample	T_g [°C][a]		
	Non-cured	105 °C	150 °C
CA0	94 ± 5.8	93 ± 2.8	99 ± 1.8
CA5	86 ± 3.1	92 ± 1.7	103 ± 1.8
CA10	85 ± 3.5	81 ± 4.1	81 ± 4.2
CA20	59 ± 0.7	55 ± 0.7	54 ± 0.5
CA30	58 ± 0.9	60 ± 0.7	58 ± 0.2

[a] Mean value of at least triplicates ± standard deviation.

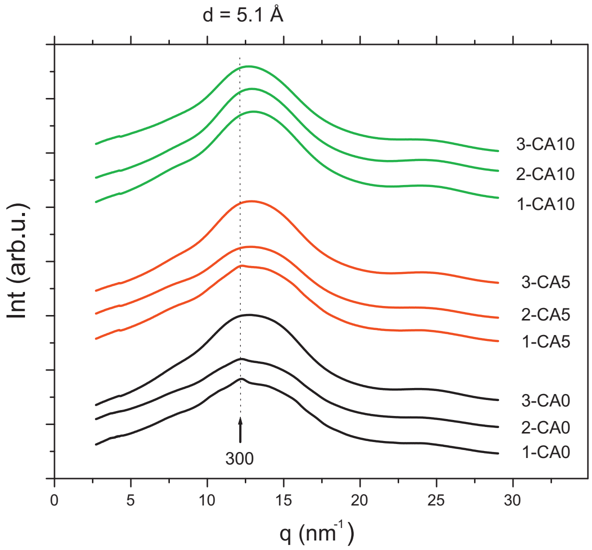

Fig. 5. WAXS patterns of starch films CA0, CA5, and CA10 without (1-) and with subsequent curing (2- 105 °C and 3- 150 °C); dotted line indicates the position of the 300 reflection of the B-type crystalline phase with d-spacing 5.1 Å.

showed a gel content of 74.0%, 75.3%, and 52.8% for films non-cured, cured at 105 °C, and cured at 150 °C, respectively. Curing at 150 °C reduced the gel content further, probably due to the strong hydrolysis of starch as seen in the M_W determination (Table 3). All starch films subjected to formic acid for 5 h at 50 °C were completely dissolved except for the film sample with 30 pph CA cured at 150 °C, which had a gel content of 8.4%. The resistance to dissolution under these tough conditions might be due to isles within the starch film with a high cross-linking density. This assumption would be consistent with earlier reports describing highly and weakly substituted regions in starch films (Klaushofer & Bleier, 1983). The results of the molecular distribution of amylose and amylopectin (Fig. 1a and b) showed that the amylopectin fraction was highly degraded with increasing CA concentration when the films were cured at 150 °C. It has been proposed in the past that small molecules or short chains are more favorable to cross-linking reactions due to more flexibility in space (Wing, 1996). In general, gel content was highly reflected by molecular structure, i.e. hydrolysis and modest cross-linking up to 20 pph CA resulting in no gel content whereas at 30 pph CA high gel content was detectable due to the stronger cross-linking.

3.7. *Starch film structure and morphology by WAXS*

The WAXS diffractograms of the starch films showed rather similar film morphology, i.e. two peaks of high intensity with average d-spacings of 4.7 Å and 2.6 Å, respectively (Fig. 5). These peaks were mainly due to the presence of an amorphous phase in the starch film structure. A small peak (Fig. 5, indicated by a dotted line) with a d-spacing of 5.1 Å was observed for the non-cured films with 0 and 5 pph CA, and the film without CA cured at 105 °C (2D data for 0 pph CA content is reported in the supplementary Fig. I). No such peak was found for the films containing 10 pph (Fig. 5), or 20 and 30 pph of CA (data not shown). The small observed peak is most likely related to the presence of B-type crystalline structure, which is typically observed for the potato starch, and is identified as the 300 reflection (Myllärinen, Buleon, Lahtinen, & Forssell, 2002; Zobel, 1988). Complementary SAXS measurements also revealed the presence of the 100 reflection (results not shown), confirming the structural identification of the residual crystalline phase. Films containing 0 to 5 pph CA seemed to have even lower crystallinity with increasing curing temperature, and curing at 150 °C resulted in a completely amorphous structure for all the films containing CA (Fig. 5). Despite the existence of some crystalline features in the present scattering data, the intensities of the scattering peaks are low and therefore the degree of crystallinity is negligible. No attempts of crystallinity quantification were done.

3.8. *Thermal analysis*

The results of the MDSC measurements are shown in Table 3. There were only small changes in T_g with CA content up to 10 pph, where the addition of CA led to anti-plasticization behavior. This is consistent with previous reports of moisture uptake at 50% RH, where films with 0–10 pph CA are shown to be in the anti-plasticized region where all the CA theoretically should be bound to the hydroxyl groups of starch. Citric acid, which has been proposed to form strong hydrogen bonds with starch, would then limit the molecular movement of the films (Yu, Wang, & Ma, 2005). There was a clear reduction in the T_g when the CA content was increased from 10 pph to 20 pph. However, there was no continuous decrease in T_g when the CA content was increased to 30 pph, which might be due to the phase separation behavior at high CA content described in a previous study (Olsson et al., 2013). The strong reduction in T_g observed at 20 and 30 pph CA could be explained with the presence of a phase with mainly free CA molecules facilitating extra movement in the material. A constant T_g for high plasticizer content has also been shown for glycerol, in which the system was phase-separated as seen by two separate T_g. Neither constant T_g with increasing plasticizer concentration nor two separate T_g was reported for the lower plasticizer contents (Lourdin, Bizot, & Colonna, 1997). However, in the case of CA it was not possible to detect any second T_g close to the location of T_g for pure CA (284 K) (Lu & Zografi, 1997) from the MDSC measurements. Curing affected the T_g of the starch films differently depending on the concentration of CA. Up to 5 pph CA, there was a significant increase in T_g with increasing curing temperature. At higher CA contents there were much smaller differences between T_g at the different curing temperatures. There is a number of possible reasons for this behavior. The increase in T_g with increasing curing temperature for the films without CA and with 5 pph CA could be explained by the decrease in moisture content (supplementary Table I), reduction of M_W due to hydrolysis (Table 1), increase in M_W with cross-linking (Table 2) and by the indicated decrease in crystallinity with increasing curing temperature (Fig. 5).

4. Conclusions

The addition of CA to starch films is a suggested method to produce starch films with enhanced water and water vapor barrier properties at high relative humidity. In this study, the effect of CA content and high curing temperature has been examined. For the first time, it was shown that no high temperature is needed to cross-link starch with CA. However, the degree of cross-linking increased with higher temperature, at least for films with 30 pph CA. Furthermore, it was seen that starch was degraded during both drying and curing when CA was present and that curing at 150 °C resulted in severe hydrolysis. We believe that this is the first study that detected and quantified CA di-ester using a modification of the complexometric titration with copper (II)-sulfate. Furthermore, a method to detect cross-linking with regard to M_W changes in the starch polymer has been described for the first time. Moreover, measurements of physical properties such as water solubility, gel content and T_g confirmed the results of the molecular analyses. The here described CA starch films are an easy way to produce cheap materials for water vapor barrier coatings for e.g. packaging material without a necessary curing step. The material showed increased water vapor barrier properties (Olsson et al., 2013) and can be used for food contact materials since CA is approved as a food additive.

Author contributions

The manuscript was written with contributions from all authors. All authors have given their approval for the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.03.044.

References

Altskär, A., Andersson, R., Boldizar, A., Koch, K., Stading, M., Rigdahl, M., et al. (2008). Some effects of processing on the molecular structure and morphology of thermoplastic starch. *Carbohydrate Polymers*, *71*, 591–597.

Andersson, R., Fransson, G., Tietjen, M., & Åman, P. (2009). Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry*, *57*, 2004–2008.

Bleier, J., & Klaushofer, H. (1983). Versuche zur Aufklärung der Struktur von Citratstärken. 2. Mitteilung. Strukturmodelle einzelner Citronensäureester der Amylose und des Amylopektins. *Starch – Stärke*, *35*, 12–15.

Carvalho, A. J. F., Zambon, M. D., da Silva Curvelo, A. A., & Gandini, A. (2005). Thermoplastic starch modification during melt processing: Hydrolysis catalyzed by carboxylic acids. *Carbohydrate Polymers*, *62*, 387–390.

Forssell, P., Lahtinen, R., Lahelin, M., & Myllärinen, P. (2002). Oxygen permeability of amylose and amylopectin films. *Carbohydrate Polymers*, *47*, 125–129.

Ghanbarzadeh, B., Almasi, H., & Entezami, A. A. (2011). Improving the barrier and mechanical properties of corn starch-based edible films: Effect of citric acid and carboxymethyl cellulose. *Industrial Crops and Products*, *33*, 229–235.

Ghosh Dastidar, T., & Netravali, A. N. (2012). 'Green' crosslinking of native starches with malonic acid and their properties. *Carbohydrate Polymers*, *90*, 1620–1628.

Godbillot, L., Dole, P., Joly, C., Rogé, B., & Mathlouthi, M. (2006). Analysis of water binding in starch plasticized films. *Food Chemistry*, *96*, 380–386.

Graffmann, G., Domels, H., & Sträter, M. L. (1974). Schnelle Bestimmung von Citrat in Wasch- und Reinigungsmitteln durch Titration mit Cu-Lösung. *Fette, Seifen, Anstrichmittel*, *76*, 218–220.

Gramera, R. E., Heerema, J., & Parrish, F. W. (1966). Distribution and structural form of phospahte ester groups in commercial starch phosphates. *Cereal Chemistry*, *43*, 104–111.

Hammersley, A. P., Svensson, S. O., Thompson, A., Graafsma, H., Kvick, E., & Moy, J. P. (1995). Calibration and correction of distortions in two-dimensional detector systems. *Review of Scientific Instruments*, *66*, 2729–2733.

Hirashima, M., Takahashi, R., & Nishinari, K. (2004). Effects of citric acid on the viscoelasticity of cornstarch pastes. *Journal of Agricultural and Food Chemistry*, *52*, 2929–2933.

Klaushofer, H., Berghofer, E., & Pieber, R. (1979). Quantitative Bestimmung von Citronensaure in Citratstärken. *Starch – Stärke*, *31*, 259–261.

Klaushofer, H., Berghofer, E., & Steyrer, W. (1978). Stärkecitrate–Produktion und anwendungs-technische Eigenschaften. *Starch – Stärke*, *30*, 47–51.

Klaushofer, H., & Bleier, J. (1982). Versuche zur Aufklärung der Struktur von Citratstärken. 1. Mitteilung. Spaltung der Esterbindung unter milden Reaktionsbedingungen. *Starch – Stärke*, *34*, 271–273.

Klaushofer, H., & Bleier, J. (1983). Versuche zur Aufklärung der Struktur von Citratstärken. 3. Mitteilung. Ergebnisse von Abbauversuchen mit amylolytischen Enzymen. *Starch – Stärke*, *35*, 237–242.

Krumova, M., López, D., Benavente, R., Mijangos, C., & Pereña, J. M. (2000). Effect of crosslinking on the mechanical and thermal properties of poly(vinyl alcohol). *Polymer*, *41*, 9265–9272.

Larson, R. G. (1999). *Polymer gels. The structure and rheology of complex fluids*. New York: Oxford University Press.

Lourdin, D., Bizot, H., & Colonna, P. (1997). Antiplasticization in starch-glycerol films? *Journal of Applied Polymer Science*, *63*, 1047–1053.

Lu, Q., & Zografi, G. (1997). Properties of citric acid at the glass transition. *Journal of Pharmaceutical Sciences*, *86*, 1374–1378.

Mammen, C. B., Ursby, T., Cerenius, Y., Thunnissen, M., Als-Nielsen, J., Larsen, S., et al. (2002). Design of a 5-station macromolecular crystallography beamline at MAX-lab. *Acta Physica Polonica A*, *101*, 595–602.

Morrison, W. R., & Laignelet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science*, *1*, 9–20.

Myllärinen, P., Buleon, A., Lahtinen, R., & Forssell, P. (2002). The crystallinity of amylose and amylopectin films. *Carbohydrate Polymers*, *48*, 41–48.

Olivato, J. B., Grossmann, M. V. E., Bilck, A. P., & Yamashita, F. (2012). Effect of organic acids as additives on the performance of thermoplastic starch/polyester blown films. *Carbohydrate Polymers*, *90*, 159–164.

Olsson, E., Hedenqvist, M. S., Johansson, C., & Järnström, L. (2013). Influence of citric acid and curing on water sorption, diffusion and permeability of starch films. *Carbohydrate Polymers*, *94*, 765–772.

Reddy, N., & Yang, Y. (2010). Citric acid cross-linking of starch films. *Food Chemistry*, *118*, 702–711.

Shi, R., Zhang, Z., Liu, Q., Han, Y., Zhang, L., Chen, D., et al. (2007). Characterization of citric acid/glycerol co-plasticized thermoplastic starch prepared by melt blending. *Carbohydrate Polymers*, *69*, 748–755.

Stading, M., Rindlav-Westling, Å., & Gatenholm, P. (2001). Humidity-induced structural transitions in amylose and amylopectin films. *Carbohydrate Polymers*, *45*, 209–217.

Wing, R. E. (1996). Starch citrate: Preparation and ion exchange properties. *Starch – Stärke*, *48*, 275–279.

Yu, J., Wang, N., & Ma, X. (2005). The effects of citric acid on the properties of thermoplastic starch plasticized by glycerol. *Starch – Stärke*, *57*, 494–504.

Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch – Stärke*, *40*, 1–7.