



## Molecular structure of citric acid cross-linked starch films



Carolin Menzel<sup>a,\*</sup>, Erik Olsson<sup>b,1</sup>, Tomás S. Plivelic<sup>c</sup>, Roger Andersson<sup>a</sup>,  
Caisa Johansson<sup>b</sup>, Ramune Kuktaite<sup>d</sup>, Lars Järnström<sup>b</sup>, Kristine Koch<sup>a</sup>

<sup>a</sup> Department of Food Science, Uppsala BioCenter, Swedish University of Agricultural Sciences, Box 7051, SE-75007 Uppsala, Sweden

<sup>b</sup> Department of Chemical Engineering, Karlstad University, SE-65188 Karlstad, Sweden

<sup>c</sup> MAX IV Laboratory, Lund University, Box 118, SE-22100 Lund, Sweden

<sup>d</sup> Department of Agrosystems, Swedish University of Agricultural Sciences, SE-23053 Alnarp, Sweden

### ARTICLE INFO

#### Article history:

Received 20 November 2012

Received in revised form 21 January 2013

Accepted 13 March 2013

Available online 21 March 2013

#### Keywords:

Starch-based film

Citric acid

Cross-linking

Molecular weight

Hydrolysis

Amorphous starch

Plasticization

### 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 distinguish 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.

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### 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

\* Corresponding author. Tel.: +46 018 672070; fax: +46 018 672995.

E-mail address: [carolin.menzel@slu.se](mailto:carolin.menzel@slu.se) (C. Menzel).

<sup>1</sup> These authors contributed equally.

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  $\mu$ L 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  $\mu$ m 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:

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

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

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