

Material Properties and Molecular Aspects of Highly Acetylated Starch-Based Films

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Received January 31, 2014; Accepted April 17, 2014

ABSTRACT: Properties of starch triacetate films from different botanical origins were evaluated. Tensile and barrier properties, glass transition temperatures and moisture uptake were measured, and the molecular structure was characterised. High-amylose starches were good film formers, normal potato starch formed continuous but brittle films and potato amylopectin formed very brittle films even after plasticisation. Barley amylopectin did not form continuous films. Different plasticisers were studied and diacetin was shown to perform better than the standard plasticiser triacetin. All films were water resistant, and the best films gave water vapour barriers in the range of films of polylactic acid, cellulose acetates and gluten. The inherent oxygen barrier of native starches was lost when the starches were highly acetylated. Starch triacetates are commonly dissolved in organic solvents such as chloroform. When testing different approaches to dissolve the starch triacetates it was found that acetic acid as well as acetylacetone were good solvents without imposing negative effects on material properties.

KEYWORDS: Highly acetylated starch, oxygen barrier, material properties, molecular characterisation

1 INTRODUCTION

For some years, the utilisation of biodegradable renewable materials to replace petroleum-based plastics has been highlighted. Starch has been considered a candidate primarily due to its price, availability and rapid biodegradation [1, 2]. The barrier of starch films against oxygen is excellent [3, 4]. It has therefore been a candidate as barrier in food packaging.

There are a few pitfalls that limit the use of starch as unsupported films. One is the variation in properties depending on humidity. The starch films become brittle in a dry atmosphere, whereas in high humidity the films lose both strength and oxygen barrier. Most native carbohydrate and protein films lose their barrier properties above relative humidity of 80%. Water sensitivity of starch can be reduced either

by modifying a majority of hydroxyl groups with, e.g., aliphatic esters [5, 6] or by grafting with larger hydrophobic molecules [1], by compounding with less hydrophilic polymers [7], by crosslinking [8, 9] or by surface modification [10].

Highly acetylated starches are thermoplastic, good film formers and resistant to water. They have been evaluated since the 40s [5, 11], but have not been developed commercially in a larger scale due to concurrency with similar cellulose derivatives which have better performance regarding film properties and cost [12]. Starch acetates become insoluble in water when 1.6 out of 3 hydroxyl groups on the glucose unit have been acetylated. In most studies fully acetylated (DS 3, triacetates) are used. To dissolve these materials prior to film casting, organic solvents such as chloroform are commonly used. Highly acetylated starch can be

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DOI: 10.7569/JRM.2014.634110

produced in reaction with acetic anhydride [13, 14]. These materials can be fully renewable and biodegradable [15]. Applications that have been evaluated are adhesives [16], dispersion coating of paper [17, 18] and coating of tablets for the pharmaceutical industries [19, 20].

Starches from different origins have diverse abilities to form films. Higher content of the linear amylose improves film properties, and longer side chains in the branched amylopectin may also enhance film properties. Native starch with 20–30% amylose, like potato starch, performs better as film former than expected. Lourdin *et al.* [21] reported that the material properties of native starches differed from blends of purified amylose and amylopectin with the same ratio of the two components. The standard starch material used to produce highly acetylated starch are commercially available high-amylose maize starch [5], which contains about 70% linear amylose.

A major disadvantage with starch films is their brittle nature and need of plasticisation, which also holds true for starch triacetates. As default, triacetin (acetylated glycerol) is used as plasticiser [5, 22–24].

There could be advantages to using highly acetylated starches rather than cellulose acetates in material applications. Since starch is easier to dissolve than cellulose, production methods could be less energy consuming. Moreover, the biological degradation of starch acetate is fast and complete [15]. New types of starches are continuously being developed, both through gene technology and by conventional plant breeding [25]. In this study, we have evaluated acetylated potato starches since the linear chains from both the mainly linear amylose, and the outer chains of the heavily branched amylopectin, are longer in tuber than in cereal starches [26].

The aim of this study was to increase the understanding of relations between different starch types and their performance as triacetates. Commonly, high-amylose maize starch has been used as standard in starch triacetate studies. We have tried to assess the importance of the length of the outer side chains of amylopectin for the performance of triacetates as films using potato starches. Increasing the knowledge about the influence of starch molecular size and chain length distribution on material properties is valuable considering that the molecular properties of starch can be optimised by gene technology. The study included film-forming ability, tensile and barrier properties as well as the molecular characterisation of highly acetylated starches with varying amylose content and with differences in amylopectin chain length. The possibility of finding better ways to plasticise and dissolve starch triacetates was explored.

2 EXPERIMENTAL

2.1 Samples

The starches used were from normal potato (np; approx. 20% amylose), amylopectin potato (pap; <1% amylose), high-amylose potato (hap; approx. 55% amylose), high-amylose maize (ham; approx. 70% amylose) and waxy barley (bap; <5% amylose), and were all kindly provided by Lyckeby Stärkelsen (Kristianstad, Sweden), except hap, which was kindly provided by PSS AB (Göteborg, Sweden). The plasticisers used were triethylcitrate, diacetin (both Merck Schuchardt OHG, Hohenbrunn, Germany), triacetin (VWR, Fontenay-sous-Bois, France), and distilled acetylated monoglyceride (EPZ) (Eastman Chemical Company, Kingsport, TN, US). All four plasticisers were food grade.

2.2 Acetylation

Prior to acetylation, the starches were hydrolysed with concentrated HCl for 16 h at 45°C in order to decrease the viscosity. Acid-hydrolysed samples were denoted with the prefix h- for *acid-hydrolysed* and ha- for *acid hydrolysed* and *acetylated*. Acetylation was conducted on starches of normal and hydrolysed potato, hydrolysed amylopectin potato, normal and hydrolysed high-amylose potato, normal and hydrolysed high-amylose maize and hydrolysed waxy barley. See Table 1 for sample abbreviations.

Triacetates were produced by the acetylation method according to Mark and Mehlretter [13]. Native or hydrolysed starch (117 g, dry weight) was refluxed in acetic acid anhydride (434 g) for 5 h at 135°C. Sodium hydroxide (22 g) was added dropwise to catalyse the reaction. The acetylated starch was allowed to cool to below 100°C and was recovered by precipitation in cold tap water during stirring. The residue was washed twice with water, dried in an oven at 50°C and finally milled with a 500 mm screen. According to Mark and Mehlretter [13], full acetylation, i.e., DS 3, is reached by this method. The reaction yield is approximately 80%.

2.3 Film Preparation

Films were prepared with and without plasticiser. In a preliminary study, we found that acetylated starches could be solved in acetylacetone, which was preferred over the standard hazardous organic solvents.

Starch (1 g) was dissolved in 15 g acetylacetone by stirring at room temperature for 15 min – 2 h, till the solution was clear. The solution was poured into a Teflon®-coated petri dish (i.d. 95 mm) and was allowed to dry for three days at 23°C.

Table 1 Visual assessment of film formation of high acetylated starches (in chloroform) plasticized with 23% triacetin

Samples	Abbreviations	Film formation ^a	Appearance ^b
Acetylated high-amylose maize	a-ham	Smooth continuous films	Transparent
Hydrolysed acetylated high-amylose maize	ha-ham	Smooth continuous films	Transparent
Hydrolysed acetylated normal potato starch	ha-np	Smooth continuous films	Transparent ^c
Acetylated high-amylose potato	a-hap	Rough continuous films	Opaque
Hydrolysed acetylated high-amylose potato	ha-hap	Rough continuous films	Less opaque
Hydrolysed acetylated potato amylopectin	ha-pap	Fractured film	Transparent
Hydrolysed acetylated barley amylopectin	ha-bap	Fragments	–

^a Ability of acetylated starches to form continuous films through solution casting

^b Optical appearance

^c Dissolved in acetylacetone

For plasticised films, the total amount of starch and plasticiser was kept constant at 1.3 g. To 1 g of starch, 0.3 g (i.e., 23%) triethylcitrate, diacetin, triacetin or EPZ was added and films were cast as described above.

To study the effect of plasticiser content on tensile and barrier properties, films were produced from selected acetylated starches with the addition of 0.3, 0.43 and 0.52 g, triacetin, corresponding to plasticiser contents of 23, 33 and 40%, respectively, based on dry weight.

All films were conditioned at 23°C and 50% relative humidity for at least 48 h before further analyses.

2.4 Material Properties

2.4.1 Tensile Tests

Tensile tests were performed according to a modified version of ASTM D882-91 using an Instron Universal Testing Machine 5542 (Instron, Norwood, MA, USA). The films were cut into 4 mm wide strips and fastened in the grips. The initial distance between the grips was approximately 30 mm and the grip separation speed was set to 2 mm/min. For each sample 6–10 replicates were made.

2.4.2 Water Vapour Permeability

Water vapour permeability (WVP) was measured according to ASTM E96-90. Cups with cylindrical wells were filled with 6 g of distilled water and covered with the sample films. The effective sample area was 5 cm². The cups were stored in a climate chamber at 23°C and 50% RH and fans were used to keep a constant flow of air over the cups. The cups were weighed at regular intervals and the water vapour permeability was

calculated according to McHugh *et al.* [27]. Samples were measured in triplicate.

2.4.3 Oxygen Permeability

Oxygen permeability was measured with an OX-tran 2/20 (MOCON, Minneapolis, MN, USA) in accordance with ASTM D3985. The measurements were performed at 23°C and 50% RH. The samples were masked using aluminum foil to give a sample area of 5 cm². All samples were measured in duplicate.

2.4.4 Glass Transition

Glass transition temperature (T_g) was measured with a Rheometrics RSAII rheometer (Rheometric Scientific, Piscataway, NJ, USA). A temperature gradient of 2°C/min was used and the temperature was adjusted according to a two-point calibration using gallium and indium. The value of T_g was chosen as the peak in phase angle. Samples were measured in quadruplicate.

2.4.5 Moisture Uptake

Moisture uptake was essentially determined according to Ghanbarzadeh and Almasi [28]. Small pieces (approx. 2.5 × 2.5 cm) of film samples, plasticised with 23% triacetin, were conditioned at 12% relative humidity (RH) using Drierite®, until weight decrease ceased. Thereafter, the samples were placed in a climate chamber conditioned with a saturated solution of K₂SO₄, giving a relative humidity of 92%. The films were weighed before and after K₂SO₄ conditioning and the weighing continued until a steady state was reached (weight difference less than 1%). Normal potato starch films and an unplasticised ha-np film were used for comparison. The measurements were performed in duplicate.

2.5 Molecular Characterisation

2.5.1 Molecular Size Distribution

Normal potato and high-amylose maize starches and their acid-hydrolysed counterparts (50 mg) were dissolved in NaOH to a final concentration of 5 mg starch/ml 0.1 M NaOH. Acetylated starches and films were also deacetylated and concomitantly dissolved in 0.5 M NaOH to a final concentration of 5 mg/ml. Acetylated potato starch dissolved readily, whereas acetylated high-amylose maize starch required a longer time for dissolution/deacetylation, up to several days. The samples were diluted with 0.01 M NaOH 1:1 and 1 ml was injected into a Sepharose CL-2B (GE Healthcare, Uppsala, Sweden) column (80 × 1.5 cm) and eluted with 0.01 M NaOH. The flow rate was 0.4 ml/min and 1 ml fractions were collected.

The molecular size distribution was determined by carbohydrate analysis by the phenol/sulphuric acid method [29] and iodine binding. In brief, an aliquot (500 ml) of every second fraction was mixed with 20 ml 20% phenol and 2.5 ml concentrated sulphuric acid, immediately followed by vigorous mixing. The fractions were left to cool and were mixed an additional two times before measuring the absorbance at 485 nm.

Iodine staining was used to detect amylose and amylopectin as described earlier [30]. To the remaining fractions, 1 ml deionised water and 100 ml I₂/KI solution (2 mg I₂ and 20 mg KI/ml) were added; the fractions were thoroughly mixed and spectra between 300 and 800 nm were taken for each fraction.

2.5.2 Molecular Weight

Molecular weight was determined by multi-angle laser-light scattering with RI detection (MALLS-RI). Measurements were essentially carried out according to Frigård *et al.* [31] on a DAWN DSP multi-angle laser-light scattering detector operating in chromatographic mode using a He-Ne laser light source (632.8 nm) (Wyatt Technology Corp., Santa Barbara, CA, USA). The MALLS-RI were connected to the following columns in series: a guard column Aquagel-OH Guard (Polymer Laboratories, Amherst, MA, USA) and size exclusion columns OH-PAK 803-HQ, 804-HQ and 806-HQ (SHOKO Co., Ltd, Tokyo, Japan). Samples were dissolved and deacetylated as described above and 75 µl was filtered and injected into the system.

3 RESULTS AND DISCUSSION

Solution cast films were produced from acetylated starches with different botanical origin and varying amylose content. Film-forming ability, tensile and

barrier properties and molecular characteristics were studied in order to increase the understanding of the relation between starch type and material properties. Triacetates of normal potato and amylose-free potato starch could not be achieved, due to a too high viscosity during processing. Viscosity was decreased by degrading the potato starch by acid hydrolysis prior to reaction with acetic anhydride.

For convenience, names for the botanical origin are hereafter used for identification of the acetylated and plasticised samples if not otherwise stated, and sample abbreviations will only be used in tables and figures (see Table 1).

3.1 Film-Forming Ability

The film-forming ability of the different acetylated starches, plasticised with 23% triacetin, was assessed visually (Table 1). The two high-amylose starches as well as normal potato starch formed smooth continuous films, and acid-hydrolysis of these starches prior to acetylation did not affect their film-forming ability. The high-amylopectin starches showed poor film-forming ability. The findings followed the same patterns as film-forming of native starch. The general view is that the linear amylose is a better film-forming component than amylopectin, due to its long and mainly unbranched structure [3, 21]. This also holds true for highly acetylated starches with elevated amylose levels, as seen in this study, and was also reported in the early days of starch acetate research [11]. Even after acid-hydrolysis prior to acetylation, the starches containing amylose in native state were still able to form continuous films. A poorer film-forming ability for amylopectin barley starch compared to potato amylopectin was shown by the higher degree of fragmentation of the latter. An explanation of this finding is that amylopectin chain length also plays a role in film formation. Molecular aspects will be further discussed below (Section 3.6).

The films formed were transparent and smooth with even surfaces, except for high-amylose potato starch, regardless of hydrolysis prior to acetylation. Films made from high-amylose potato starch were opaque, but the opacity was reduced by acid-hydrolysis prior to acetylation. However, even after hydrolysis, the surfaces of the high-amylose potato films were rough and uneven and are believed to be caused by incomplete dissolution of the granules or amylose aggregates. Similar observations were reported by Thunwall *et al.* [32].

3.2 Tensile Properties

Tensile testing (strain at break and stress at break) was performed on films of acetylated samples of normal

potato and high-amylose maize starch after conditioning. These samples were chosen based on the fact that they are commercially important industrial raw materials. The high-amylose potato starch sample could not be completely dissolved and tensile measurements on films containing a considerable amount of granule remnants or aggregates were considered to give unrepresentative results. In addition, no tensile testing could be performed on films of the amylopectin starches. The potato amylopectin films were too brittle to perform tests and the barley amylopectin films were too fractured for measurements (Table 1).

3.2.1 Starches with Different Botanical Background

A comparison of potato starch on one hand and high-amylose maize starch on the other hand showed that the hydrolysed and acetylated samples of both starches were comparable regarding tensile properties (Fig. 1), and that the unhydrolysed high-amylose maize stood out by showing significantly higher strain at break. The hydrolysed sample (ha-ham) exhibited markedly higher lower strain at break. Wolff *et al.* [33] studied amyloses (not acetylated) prepared from corn starch with different degrees of molecular degradation and found that film properties were lost below a certain degree of degradation. This will be discussed in Section 3.6.

3.2.2 Type of Plasticiser

The influence of four different plasticisers (23%) on tensile properties was studied for films of high-amylose maize and hydrolysed potato starch (Fig. 2). Generally, EPZ differed considerably from the other

three and showed poor plasticisation properties. For all plasticisers, high-amylose maize films showed significantly higher elongation values than potato films (Fig. 2a). A tendency for higher elongation values for diacetin for both potato and high-amylose maize starch was seen compared to triacetin. These compounds are both acetylated glycerol and differ only in one acetate group. A trend among the plasticisers (besides EPZ) seemed to indicate that they gave stronger films with increasing molecular size of the plasticiser, i.e., in the order diacetin < triacetin < triethylcitrate, but a significant difference was only detected between diacetin and triethylcitrate.

For EPZ, the pattern was different from the other plasticisers (Fig. 2b). For potato films stress at break

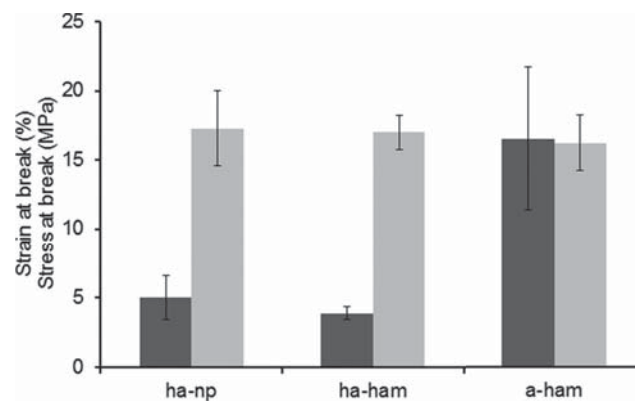


Figure 1 Strain at break (dark grey bars) and stress at break (light grey bars) for solution-cast films of ha-np, ha-ham and a-ham, plasticised with 23% triacetin. Error bars show the confidence interval at 95% significance.

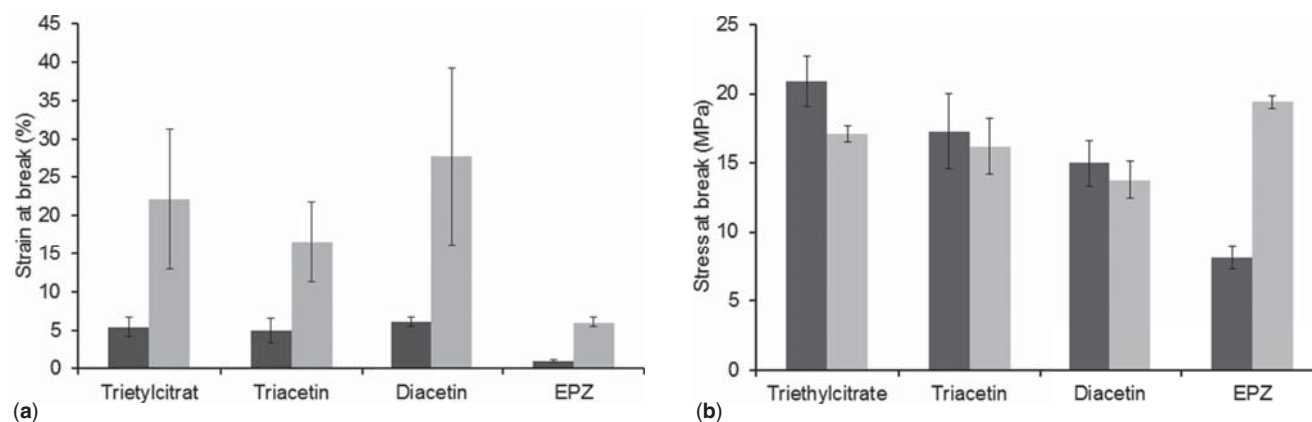


Figure 2 Tensile properties for solution-cast films of ha-np (dark grey bars) and a-ham (light grey bars) containing different types of plasticiser (23%). (a) Strain at break, (b) stress at break. Error bars show the confidence interval at 95% significance.

was considerably lower with EPZ than with any of the other plasticisers, whereas for high-amylose maize the stress at break was significantly higher. Since EPZ is an acetylated monoglyceride, it is likely that its lipid moiety can complex with amylose and possibly also with longer amylopectin chains. The plasticising effect would therefore be negligible since EPZ would be situated within the glucose helix, stabilizing the system, rather than between the starch chains for plasticisation. Concomitantly, the stabilised amylose chains would render stronger films, as was also seen by the higher stress at break for high-amylose maize with EPZ. Bader and Göritz [34] reported lower elongation for native corn starch materials as compared to defatted corn starch and attributed this effect on tensile properties to the amylose-lipid complex.

3.2.3 Amount of Plasticiser

The effect of plasticiser content on tensile properties was investigated for potato starch films plasticised with 23, 33 and 40% (w/w) triacetin. At higher triacetin contents than 40% the films were very sticky. As expected, an increasing amount of plasticiser decreased the stress at break significantly with a concomitant increase in strain at break (Table 2), even though no significant differences were seen in elongation between 23 and 33% triacetin.

3.3 Glass Transition

Glass transition temperatures of the films are given in Table 3. Plasticisation with diacetin gave films

with lower T_g than with triethylcitrate and triacetin for both potato and high-amylose maize films. The difference was significant when comparing diacetin to triacetin in potato films and triethylcitrate in high-amylose maize films. In fact, for the potato films, triacetin seemed to be the least suitable plasticiser of the three, considering glass transition temperature. Hence, these measurements supported the results on tensile properties discussed in Section 3.2.2, where a tendency towards better plasticisation properties was indicated for diacetin (Fig. 2), but which was not significantly validated. The poor plasticising ability of EPZ mentioned earlier was also demonstrated by the significantly higher T_g for both potato and high-amylose maize films. As anticipated, T_g decreased noticeably with increasing plasticiser content, and the results were congruent with the changes in tensile properties (Table 2).

Table 2 Tensile properties for films of acid-hydrolysed and acetylated potato starch with different content of triacetin (with confidence interval at 95% significance)

Triacetin (%)	Stress at break (%)	Strain at break (MPa)
23	17.3 ± 2.7	5.0 ± 1.6
33	11.6 ± 0.8	3.4 ± 0.4
40	1.5 ± 0.2	49.9 ± 3.5

* Confidence interval at 95% significance.

Table 3 Glass transition of highly acetylated starches (in acetylacetone) plasticized with different plasticisers to different extent (confidence interval at 95% significance)

Sample	Plasticiser	Content (%)	T_g (°C)	M_w (10^6 g/mol)	M_n (10^4 g/mol)
ha-np	Triethylcitrate	23	83.1 ± 2.45		
ha-np	Triacetin	23	97.8 ± 0.00	5.68 (± 0.34)	0.50 (± 0.60)
ha-np	Diacetin	23	79.4 ± 1.57		
ha-np	EPZ	23	112.7 ± 0.20		
ha-np	Triacetin	33	63.8 ± 0.20*		
ha-np	Triacetin	40	54.2 ± 1.30*		
a-ham	Triethylcitrate	23	87.4 ± 1.47		
a-ham	Triacetin	23	87.1 ± 2.06	1.04 (± 0.22)	8.06 (± 1.20)
a-ham	Diacetin	23	83.4 ± 2.35		
a-ham	EPZ	23	118.1 ± 1.08		
ha-ham	Triacetin	23	79.3 ± 1.27	0.16 (± 0.01)	4.21 (± 2.30)

* Duplicates.

3.4 Moisture Uptake

Films were preconditioned and subjected to 92% relative humidity, and moisture uptake was measured gravimetrically for starch triacetates from high-amylose maize and potato starch and compared to unmodified potato films. Unplasticised films from potato triacetates were also studied (Table 4). The main finding was that films of triacetates were almost insensitive to moisture, whereas films based on native potato starch, as expected, absorbed moisture to a much larger extent. Higher moisture uptake was found for

acetylated potato films over films made from both high-amylose maize starch films.

Possibly, the presence of more amylopectin and perhaps also phosphate groups, if still present after the acetylation, in potato than in high-amylose maize facilitate the absorption of water. In addition, the unplasticised acetylated potato films absorbed significantly more moisture than its plasticised counterpart. This phenomenon can potentially be explained by a tighter structure in films *with* plasticiser. Triacetin itself is partially water soluble, and this suggests that triacetin has an effect on film structure.

Table 4 Moisture uptake of solution-cast films of starch triacetates

Sample	Moisture uptake (%)
ha-np (23% triacetin)	2.56 ± 0.12
ha-np (33% triacetin)	
a-ham (23% triacetin)	1.84 ± 0.28
ha-ham (23% triacetin)	1.79 ± 0.08
ha-np (no plasticiser)	4.18 ± 0.55
np (no plasticiser)	15.9 ± 4.79
np (62% glycerol)	38.9

3.5 Barrier Properties

The low moisture uptake values were matched by low water vapour permeability for the acetylated films. The WVP varied between 0.7 and 0.8 g × mm/m²h × kPa for high-amylose maize and potato starch triacetate films, which is lower than for other biopolymeric films such as proteins and polysaccharides (Table 5); even lower than cellulose triacetate [35]. The highest WVP was seen for potato films containing elevated levels (33%) of triacetin. This result is in contrast to the finding that plasticised potato films absorbed less moisture than unplasticised ones. It is known that the barrier properties of polymer films often are lost with plasticisation due to a higher mobility of the smaller plasticising molecule [36].

As opposed to the high water vapour barrier, oxygen barrier was poor for all triacetate films. Since

Table 5 Water vapour and oxygen permeability properties of solution-cast films of starch triacetates (confidence interval at 95% significance). References are given for barrier properties measured by other groups

Sample	WVP (g×mm/m ² h×kPa)	OP (cm ³ ×µm/m ² ×d×kPa)	Reference
ha-np (23% triacetin)	0.8 ± 0.11	792	
ha-np (33% triacetin)	1.3 ± 0.34	1441	
a-ham (23% triacetin)	0.7 ± 0.11	Over range	
ha-ham (23% triacetin)			
ha-np (no plasticiser)			
np (no plasticiser)			
np (62% glycerol)			
Amylose acetate		192	[27]
Cellulose acetate	2.4 - 24	100 - 1 000	[25]
PLA	2.4 - 24	100 - 1 000	[25]
Gluten	2.4 - 24	1 - 10	[25]
Amylose (glycerol)	5.0 - 103	7	[3, 28]
Proteins	7 - 120	0.7 - 43	[29]

* Confidence interval at 95% significance.

acetylation decreases the polarity, and the hydroxyl groups are highly polar, a loss of barrier would be expected. Decreasing polarity of substituents that were added to the same carbon backbone increased oxygen permeability by five orders of magnitude [37].

The plasticisation with triacetin also increased oxygen permeability. In general, the high OP values were in the same range as found for films of unplasticised amylose acetate [38, 39], cellulose acetate and polylactic acid [35]. For the high-amylose maize film, OP reached the measuring limit and hence could not be assessed.

3.6 Molecular Aspects

The molecular size distribution of the acetylated samples and their native counterparts are shown in Figure 3. The first peak in the chromatograms represents the amylopectin and the second broad peak the amylose and also the degradation products. The scattered marks represent the wavelength of maximum absorbance in the iodine-stained fractions (λ_{\max}), and give an indication of the glucose chain length up to a degree of polymerisation (DP) of approx. 400 [40, 41]. In general terms, amylose shows λ_{\max} values above 600, with a maximum at around 640 nm, and amylopectin has a characteristic λ_{\max} between 540 and 580 nm.

A significant degradation of the amylopectin was found in the triacetate of the potato sample (Fig. 3a), as would be expected due to the acid-hydrolysis prior to acetylation. The amylopectin peak was reduced and the degraded amylopectin molecules eluted at higher elution volume together with degraded amylose, seen as a distinctive peak in the chromatogram. Also, a reduction in λ_{\max} could be observed over the amylopectin

peak as well as over the first part of the amylose region and the last part of the chromatogram. The former is indicative of a reduction of average amylopectin chain length. The latter two can be explained by fragmentation of the amylopectin into domains of clusters as well as to a degradation of high-molecular weight amylose [30], leading to later elution of the fragmented molecules and a concomitant decrease in average λ_{\max} .

Regarding high-amylose maize, acetylation without prior acid-hydrolysis only led to minor degradation of the raw material, as shown by the slightly altered chromatographic profile and lower λ_{\max} values of a-ham compared to ha-ham. A minor degradation by acetylation process has also been described by Mark and Mehlretter [13] and Shogren [42]. Acid-hydrolysis had, as would be anticipated, a strong impact on high-amylose maize (Fig. 3b), which was completely deprived of high-molecular weight amylopectin. Likewise, high-molecular weight amylose was degraded and only one major peak with a maximum at 123 ml was detected. The strong degradation is further seen by the total absence of λ_{\max} values of the elution volume up to 98 ml and by the markedly lower λ_{\max} over the amylose peak (elution volume 100–120 ml). In an early study, Wolff *et al.* [33] showed that amylose degradation to a critical degree of below DP 230 had a critical effect on film formation. In terms of molecular weight (Table 3), this corresponds to approx. 0.37×10^5 g/mol. The measured M_w of hydrolysed high-amylose maize starch was below this limit (0.16×10^5 g/mol), but despite that, coherent films were obtained in our study, which would imply that the acetate groups facilitated the formation of films. The material properties in the study of Wolff *et al.* [33] were only slightly affected at a degree of degradation above DP 230. This implies that it could be possible to degrade the high-amylose

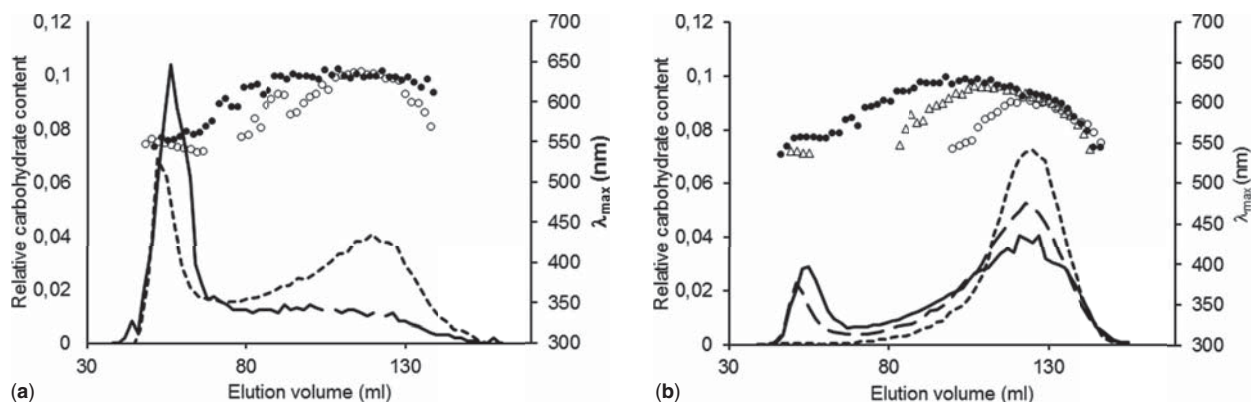


Figure 3 Chromatograms for starches fractionated on Sepharose CL-2B and detected by carbohydrate (lines) and chain length analysis (marks; as indicated by iodine staining). **(a)** Native potato starch (np: solid line and \bullet), acid hydrolysed and acetylated potato starch (ha-np: dotted line and \circ); **(b)** native high-amylose maize starch (ham: solid line and \bullet), acetylated high-amylose maize starch (a-ham broken line and Δ) and acid hydrolysed and acetylated high-amylose maize starch (ha-ham: ha-np: dotted line and \circ).

maize starch without losing properties. Starch degradation prior to processing is often an advantage in processing and increases degradability of the material.

There was a significant difference in molecular weight between the two starch types, which supported the results from the SEC analyses (Table 3). The highest average molecular weight (M_w) was found for the potato sample, due to the considerably higher amount of high-molecular weight amylopectin than the other samples (Fig. 4). The lowest M_w was found for the hydrolysed high-amylose maize triacetate. Regarding the number average molecular weight (M_n), a-ham showed the highest values, whereas ha-np showed the lowest. This implies that the degraded potato sample was composed of more strongly degraded starch molecules than the high-amylose maize samples.

It is well known that there are significant differences in the molecular structure between potato starch and high-amylose maize starch [43]. The pronounced dissimilarities in molecular structure between the native samples (Figs. 3a and 3b) were expected to influence the film-forming and material properties of films produced from these starches. The acetylated high-amylose maize starch films showed both high elongation and high stress at break, whereas films from potato and the acid-hydrolysed triacetate of maize had comparable tensile properties (Table 1) with low elongation and high strength. This indicates that the most suitable molecular structure for the formation of films with good material properties is comprised of essentially undegraded amylose and a minor content of amylopectin. The low elongation of the potato films could be attributed to the presence of a higher level of amylopectin. The entanglement of linear chains is imposed by the branched amylopectin and perhaps prevented the creation of flexible films.

The outer linear chains of the branched amylopectin are longer in potato amylopectin than in maize starch,

and the outer chains of barley amylopectin are even shorter than in maize [26]. In Table 1 it is shown that potato amylopectin formed brittle films and barley amylopectin did not form films at all. Whistler and Hilbert [11] showed that plasticised triacetates of maize starch and purified maize amylopectin were too brittle to perform measurements on. However, the potato amylopectin films seemed to be unaffected by triacetin, whereas the derivatives from normal potato starch, containing 20% amylose, did respond to plastication by triacetin.

Similar material properties were found for the films of hydrolysed potato starch and high-amylose maize starch. Only strongly degraded amylopectin and amylose were present in the latter sample, as seen by the late elution volume and the relatively low values of λ_{max} (< 605 nm) (Fig. 3b). As mentioned earlier, hydrolysed amylopectin starches were not able to form continuous films, due to the predominant presence of amylopectin fractions of low molecular weight, or expressed from the view of amylose, the absence of enough linear chains of suitable length intact for film formation. Although the acid-hydrolysis severely degraded the high-amylose maize starch, sufficient linear amylose was left so that film formation could take place.

3.7 Type of Solvent

Acetic acid (HAc) is formed during the acetylation process, and hence the modified starch is dissolved in the acid. Therefore, HAc was tested as solvent in the solution casting process for the potato and the high-amylose maize triacetates, and tensile properties were measured. Solution casting succeeded well, and the film formation ability and appearances of the films were similar to films prepared in acetylacetone (Table 1). The tensile properties are presented in Figure 4. The films cast in HAc showed significantly higher stress at

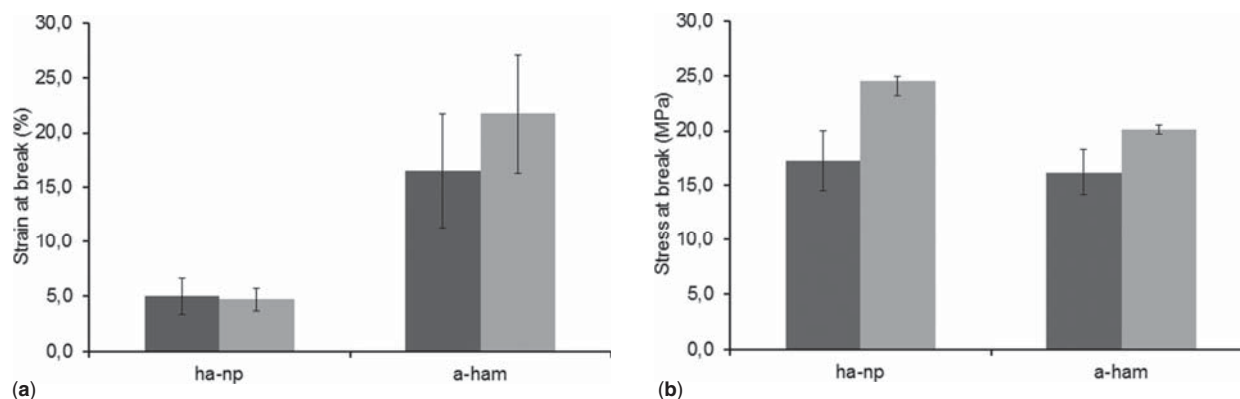


Figure 4 Tensile properties for films (ha-np and a-ham) prepared in acetylacetone (dark grey bars) and acetic acid (light grey bars). (a) Strain at break; (b) stress at break. Error bars show the confidence interval at 95% significance.

break than the corresponding films in acetylacetone. However, no difference was seen in elongation for films prepared by the same raw material in either acetylacetone or HAc (compare with Fig. 1). The results suggest that HAc can be used as an efficient solvent for highly acetylated starches, leading to materials with improved elongation properties. Furthermore, conventional hazardous solvents for starch acetates, as for instance chloroform, can be avoided in the future without having to compromise material properties.

4 CONCLUSION

Solution cast films were produced from triacetates of starches with different botanical origin and varying molecular structure. Four types of plasticiser and two solvents were investigated in order to enhance material properties. Starches with normal and high amylose content formed continuous films, whereas high-amylopectin potato starch formed poor films and barley amylopectin did not form films. Acetylated high-amylose maize starch gave the best materials when taking into consideration both their high elongation and high strength. Less flexible but strong films were formed by triacetates of hydrolysed potato and hydrolysed high-amylose maize starch. Diacetin appeared to be a better plasticiser than the conventional triacetin, as seen by lower glass transition temperatures as well as by a trend towards higher strain at break for diacetin plasticised films.

Moisture uptake and barrier properties were measured for films containing 23% triacetin. Starch triacetates were shown to have little uptake of moisture. The oxygen barrier was lost when the hydrophobicity was increased through acetylation, which shows that one cannot expect to retain the oxygen barrier when water resistance is improved. Starch triacetates were soluble in acetic acid. Tensile properties were not impaired compared to films cast from conventional organic solvents. This can open up new possibilities for starch triacetates.

5 ACKNOWLEDGMENTS

The authors thank Lyckeby Starch for providing starch samples and for valuable discussions and Plant Science Sweden AB for providing the high-amylose potato starch. This study was performed within the project Renewable Functional Barriers, which is a part of the BFP Research Program, jointly launched by the Swedish Governmental Agency for Innovation Systems (VINNOVA), the Swedish Forest Industries Federation and Trä- och Möbelföretagen (TMF). The authors acknowledge VINNOVA and the industrial

companies taking part in the project for their financial support.

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