Production of plasticized thermoplastic starch by spray drying
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Chapter 5

Malic acid as plasticizer for thermoplastic starch?

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(To be submitted)
Abstract

Retrogradation of amorphous thermoplastic starch (TPS) films obtained by compression molding of spray dried oxidized amylopectin potato starch was investigated. The aim of the work was to investigate the influence of natural plasticizers with similar molecular weight but different hydrogen bonding functionality, i.e. isoleucine, asparagine and malic acid, on the performance of powder and TPS films. Combinations of malic acid with glycerol, urea, and maltodextrin were also evaluated.

Except for isoleucine formulated starch, all samples were obtained and evaluated as amorphous powders and films. Malic acid was identified as a strong anti-retrogradation agent as it inhibited recrystallization of starch for 56 days over the full range of relative humidity levels. Malic acid also was found to inhibit the retrogradation of mixed formulations containing urea, glycerol and maltodextrin. The converse of the strong inhibition implied strong moisture absorption and high strain at break values, with low tensile strengths. Malic acid was also identified as a potential cross-linking agent to control swelling of starch-based products.

Asparagine and isoleucine formulated starch films showed no retrogradation at RH0%. At RH50% and RH100% significant retrogradation occurred. Hydrophobic isoleucine exuded from the starch matrix and re-crystallized during spray drying. Asparagine was found to lower the T_g of the starch matrix and as a major contributor to the crystallinity of asparagine plasticized TPS films.

Introduction

Retrogradation of starch in TPS based products is a major issue that needs to be controlled in order to develop new starch based products and to add product value. This because upon retrogradation, fractions of the polymeric starch chains rearrange and form crystalline domains [1, 2], which alters the physical and mechanical properties of the material and reduces the number of practical applications. Similar to synthetic polymers, the increase in crystallinity leads to an increased modulus, a decrease in strain, and brittleness; while the absence of retrogradation may lead to uncontrolled moisture absorption and loss of mechanical properties. Therefore, reducing the dynamic behaviour of the material by means of physical and chemical treatment or the addition of plasticizers to improve starch processing and to control mechanical properties is studied. Besides low molecular weight hydrogen bonding plasticizers, also water acts as a plasticizer in starch [3, 4]. Therefore the rate of moisture uptake and the absolute level of moisture uptake are also important factors affecting mechanical and retrogradation properties [4-7].

Several studies focussed on the effect of different plasticizers on TPS films [8]. Polyols, such as glycerol, xylitol and sorbitol closely resemble the starch structure and are therefore widely used [7, 9-11]. Likewise, citric acid was shown to act as plasticizer and also inhibited re-crystallization of starch [12]. Many of the tested chemicals indeed plasticize the starch matrix, but it is remarkable to note that there is a large variation in the retrogradation behaviour and moisture uptake for the different formulations. Glycerol and some low molecular weight amide structures (i.e. urea or formamide) are proven to plasticize starch, of which the amides perform significantly better in suppressing retrogradation. The latter, however, is accompanied with more extensive moisture absorption [13-15]. The effectiveness of amide structures and citric acid in suppressing the retrogradation has been attributed to efficient hydrogen bonding or chemical reaction [16]. In
addition, literature clearly showed a higher effectiveness for low molecular weight plasticizers in suppressing retrogradation [10, 13, 14].

In previous chapters 3&4, amorphous TPS films were produced via compression molding of solution spray-dried powder. In the first part, maltodextrins of different molecular weights were studied to evaluate whether they act as plasticizer or processing aid. It was observed that in the dry starch formulation as made by the spray-drying process, the additives did not act as plasticizer, while the rate of moisture uptake appeared to be the highest for the lowest molecular weight additive with an opposite trend for the rate of recrystallization. In the next chapter 4, natural plasticizers, i.e. glycerol and urea, were used separately and in combinations with maltodextrin; this to investigate the rate of retrogradation of compression molded films compared to solution casted ones. It was found that glycerol and urea acted as effective plasticizers, i.e. lowered the $T_g$ of the system, but the retrogradation behaviour differed significantly. Upon exposure to high humidity conditions, glycerol stimulated rapid retrogradation while urea delayed this process but promoted moisture absorption. The addition of maltodextrin again promoted retrogradation for both combinations.

Against this backdrop a study was undertaken with the aim to generate better understanding about the interaction of low molecular weight and hydrogen bonding additives during and after the spray-drying of starch. Three potential plasticizers with almost identical molecular weight but containing different hydrogen bonding functionality were chosen (see Figure 1). In the first part of the study isoleucine, asparagine and malic acid containing carboxyl, amino, amide and hydroxyl functional groups were evaluated as plasticizers. To the best of our knowledge, these additives were never evaluated as starch plasticizer, not in classic processing operations such as sheet extrusion or film blowing, nor in the spray-drying of starch.

![Figure 1](image)

**Figure 1** Natural plasticizers, having similar molecular weights.

In the second part of the study combinations of malic acid and the more traditional plasticizers like glycerol, urea and maltodextrin were evaluated. All starch-plasticizer mixtures were formulated as aqueous solutions and evaluated as amorphous spray-dried powders and compression molded films. For the powders the particle morphology, thermal properties and molecular interactions between the powder components was tested by SEM, TGA and DTGA, and FT-IR analysis, respectively. For the films retrogradation, moisture uptake and mechanical properties were the main points of focus.
Materials and Methods

Materials

Oxidized amylopectin-rich potato starch (Perfecta-film X85; moisture content 15%) was a gift from AVEBE (The Netherlands). Analytical grade glycerol, urea, L-isoleucine (reagent grade), L-asparagine (≥ 98%) and DL-malic acid (99%) were purchased from Sigma-Aldrich. Maltodextrin (DE = 19.1) was kindly supplied by Cargill (France). Codes for the starch formulations are depicted in Table 1. The percentage in the code name indicates the weight percentage of plasticizer used of dry starch weight.

Preparation of TPS-powder

TPS powder was prepared as follows. An aqueous solution of starch was prepared by heating 15% (w/w) of oxidized starch with different plasticizer contents. 20, 30, 40 or 15/15 % (w/w based on dry starch) of plasticizer were added to the starch solutions and heated to 95°C for 25 minutes applying a stirring rate of 600 RPM to prepare homogeneous solutions. The obtained solutions were fed to a Buchi mini spray drier B-191 equipped with a 0.7 mm nozzle. The procedure to produce amorphous powder was adopted as described in previous chapters 2&3.

Preparation of TPS-films

TPS films (Ø10 mm x 0.5 mm) were produced by compression molding the TPS powder, using a Fontijne Holland Table Press TH400. Samples were compressed at 25 bar for 5 minutes. The molding temperature for all samples was 140°C (chapter 4).

Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Description (w/w of starch)</th>
<th>MW [g/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S</td>
<td>Feedstock material</td>
<td>-</td>
</tr>
<tr>
<td>S.D</td>
<td>Spray dried starch</td>
<td>-</td>
</tr>
<tr>
<td>20I.PS</td>
<td>20% isoleucine plasticized starch</td>
<td>131</td>
</tr>
<tr>
<td>30I.PS</td>
<td>30% isoleucine plasticized starch</td>
<td>131</td>
</tr>
<tr>
<td>20A.PS</td>
<td>20% asparagine plasticized starch</td>
<td>132</td>
</tr>
<tr>
<td>30A.PS</td>
<td>30% asparagine plasticized starch</td>
<td>132</td>
</tr>
<tr>
<td>40A.PS</td>
<td>40% asparagine plasticized starch</td>
<td>132</td>
</tr>
<tr>
<td>20MA.PS</td>
<td>20% malic acid plasticized starch</td>
<td>134</td>
</tr>
<tr>
<td>30MA.PS</td>
<td>30% malic acid plasticized starch</td>
<td>134</td>
</tr>
<tr>
<td>40MA.PS</td>
<td>40% malic acid plasticized starch</td>
<td>134</td>
</tr>
<tr>
<td>MA.G.PS</td>
<td>15% malic acid, 15% glycerol plasticized starch</td>
<td>-</td>
</tr>
<tr>
<td>MA.U.PS</td>
<td>15% malic acid, 15% urea plasticized starch</td>
<td>-</td>
</tr>
<tr>
<td>MA.MD.PS</td>
<td>15% malic acid, 15% maltodextrin plasticized starch</td>
<td>-</td>
</tr>
</tbody>
</table>
Scanning Electron Microscopy (SEM)

A Jeol 6320F Scanning Electron Microscope was used for morphological characterization. The applied accelerating voltage for measurement was 3.0 kV. Before analysis, the samples were covered with a thin palladium/platinum conductive layer created with a Cressington 208 sputter coater.

X-Ray Diffraction (XRD)

The crystalline structure of the spray dried powders and films were elucidated using XRD. A Bruker D8 equipped with Cu radiation exhibiting a wavelength of 1.5418 Å was used to record diffractograms from 5° 2θ to 40° 2θ. A step size of 0.02° 2θ using a scan speed of 2 s/step was employed. The system was operated at 40 kV and 40 mA.

Powder was analyzed using a sample holder with a sample compartment of Ø25 mm x 2.0 mm. TPS films (Ø10 mm x 0.5 mm) were analyzed using an adjustable sample holder.

Thermal Gravimetric Analysis (TGA)

Open pan TGA was performed in a Perkin Elmer TGA 7.5-10 mg. The procedure applied to study the weight change as a function of temperature was described in the previous chapter 3. The decomposition temperature (T_{dec}) was determined using the method applied by Soliman et al. (1997) [17].

Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g) of the samples was determined by DSC measurements, using a DSC-60 Shimadzu Co. Prior to analysis a reference was constructed using an empty aluminium pan. Samples were scanned at a rate of 10°C/min from 10-200°C. Open pan measurement was performed in order to remove any remaining water from all samples in the first run.

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared analyses were performed to investigate any structural changes that occurred in the starch-chain due to interaction of the plasticizer with the starch molecule. The spectra were recorded using a Perkin Elmer FT-IR spectrometer spectrum 2000 equipped with an attenuated total reflection (ATR) unit. For each spectrum 32 consecutive scans at 4 cm^{-1} resolution were averaged. All analyses were performed at ambient temperature.

Retrogradation and Moisture Uptake

Retrogradation of TPS films (Ø10 mm x 0.5 mm) was measured under different relative saturation humidity: 0%RH (dried silica), 50%RH (35.64%CaCl₂ solution) and 100%RH (distilled water). The samples were analyzed by XRD for changes in crystallinity at day 0 (directly after production) and after 7, 14, 28 and 56 days. Identical analytical conditions were applied as described in section XRD.
Chapter 5

The degree of crystallinity was determined using the method described by Wang et al. (2005) [18]. The procedure used for integration and calculation of crystallinity was explained in previous chapters 3 & 4. To study film moisture uptake the films, stored at 0%, 50% and 100% RH, were gravimetrically analyzed. Measurements were performed every day during the first seven days. Hereafter measurements were performed at day 7, 14, 21, 28 and 56. Moisture uptake of the films was determined using equation 1 [19].

\[
\text{moisture uptake} = \left( \frac{\text{weight}_{\text{day},n} - \text{weight}_{\text{day},0}}{\text{weight}_{\text{day},0}} \right) \times 100\%
\]  

(Eq. 1)

The subscript ‘day,n’ denotes the weight at the day of measurement, ‘day,0’ indicates film weight directly after film making. Moisture adsorption data were fitted according to the Peleg model (equation 2) except malic acid plasticized and co-plasticized formulations [20]. Malic acid plasticized and co-plasticized formulations were fitted using a modification of the Peleg model (Equation 3).

\[
M_t = M_0 + \left( \frac{t}{k_1 + k_2 t} \right)
\]  

(Eq. 2)

\[
M_t = M_0 + \left( \frac{t}{c_0 + c_1 t + c_2 t^2} \right)
\]  

(Eq. 3)

Table 2
Constant values (k_i, c_0, c_1, and c_2) and regression coefficient (R^2) for sorption curve equations (2 & 3) (Peleg model) of TPS films at 100RH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>M_0</th>
<th>k_1</th>
<th>k_2</th>
<th>c_0</th>
<th>c_1</th>
<th>c_2</th>
<th>R^2</th>
</tr>
</thead>
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<tr>
<td>S.D</td>
<td>5.2</td>
<td>4.2*10^{-2}</td>
<td>1.5*10^{-2}</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.99</td>
</tr>
<tr>
<td>30I.PS</td>
<td>2.5</td>
<td>3.4*10^{-2}</td>
<td>1.8*10^{-2}</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.99</td>
</tr>
<tr>
<td>30A.PS</td>
<td>2.4</td>
<td>4.1*10^{-2}</td>
<td>1.1*10^{-2}</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.99</td>
</tr>
<tr>
<td>20MA.PS</td>
<td>1.3</td>
<td>---</td>
<td>---</td>
<td>1.7*10^{-2}</td>
<td>1.2*10^{-2}</td>
<td>-9.2*10^{-5}</td>
<td>---</td>
</tr>
<tr>
<td>30MA.PS</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>2.6*10^{-2}</td>
<td>6.3*10^{-3}</td>
<td>-6.4*10^{-5}</td>
<td>---</td>
</tr>
<tr>
<td>40MA.PS</td>
<td>0.9</td>
<td>---</td>
<td>---</td>
<td>1.7*10^{-2}</td>
<td>8.2*10^{-3}</td>
<td>-6.2*10^{-5}</td>
<td>---</td>
</tr>
<tr>
<td>(15:15)MA.G.PS</td>
<td>0.5</td>
<td>---</td>
<td>---</td>
<td>1.2*10^{-2}</td>
<td>5.4*10^{-3}</td>
<td>-5.5*10^{-5}</td>
<td>---</td>
</tr>
<tr>
<td>(15:15)MA.U.PS</td>
<td>0.5</td>
<td>---</td>
<td>---</td>
<td>1.1*10^{-2}</td>
<td>5*10^{-3}</td>
<td>-6.4*10^{-5}</td>
<td>---</td>
</tr>
<tr>
<td>(15:15)MA.MD.PS</td>
<td>0.8</td>
<td>---</td>
<td>---</td>
<td>9.5*10^{-3}</td>
<td>8.9*10^{-3}</td>
<td>-1*10^{-4}</td>
<td>---</td>
</tr>
<tr>
<td>30MD.PS</td>
<td>2</td>
<td>2.8*10^{-2}</td>
<td>1.4*10^{-2}</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Where M_t is the moisture contents at time t, M_0 is the initial moisture content and k_1 is the Peleg rate constant [t/(g water/g solids)] and k_2 is the Peleg capacity constant (g solids/g water) [21]. The
Peleg parameters [20], \( k_1 \) and \( k_2 \) are shown in Table 2. The constants \( k_1 \) and \( k_2 \) are related to mass transfer and maximum water adsorption capacity, respectively. Low values for \( k_1 \) and \( k_2 \) correspond to a high initial water adsorption rate and a high adsorption capacity, respectively [21]. The \( R^2 \) values were found to be very high in all cases (\( R^2 > 0.99 \)). The original Peleg model is not sufficient for malic acid plasticized and co-plasticized formulations in terms of regression coefficient (\( R^2 < 0.99 \)); these data were modeled using a polynomial expression (Equation 3).

**Mechanical Testing**

The mechanical properties include the tensile strength (TS) and percentage elongation at break (%E). Five or more specimens were tested using an Instron 4301 tensile tester in accordance with ASTM D1708. The crosshead speed used was 10 mm/min. The samples were conditioned at 25°C and 100% RH for 24 hr and the thickness and width of the samples were measured before testing.

**Results and Discussion**

In previous chapters 3&4, it has been shown that the effective mixing of starch and plasticizer followed by drying and film production resulted in film performance different from films produced by classic extrusion technology or solvent casting. It further was found that this interaction of plasticizers and starch in the spray-dried formulations has a positive effect on the rate of retrogradation and the rate of moisture uptake. The behavior of compression molded films in terms of moisture absorption (at different humidity levels) and rate of retrogradation was found to depend on the type of plasticizer and the presence of maltodextrin as a third formulation component. In this research, natural plasticizers i.e. isoleucine, asparagine, and malic acid with similar molecular weight were used to study the effect of molecular structure on the rates of retrogradation and moisture uptake of TPS films.

**Powder Morphology**

Figure 2 shows the granular morphology of the spray dried powder samples. In line with previous work (chapter 3&4), the feedstock material exhibited spherical and oval shaped particles of irregular sizes. The surface of the particles was homogeneous.

A white powder was obtained for all samples after spray drying. SEM analyses showed visibly different particle morphology after solution spray drying (Figure 2). Morphology of powder granules was similar to previous chapters 3&4. Particles were also spherical in nature however they contained dents, which is typical for spray dried powders [22, 23]. The degree of shrinkage is determined by the drying sequence [24, 25], which leads to the formation of dents in the particle surface (chapter 2). The deformation of the particles after spray drying also indicates the possible formation of hollow particles [25].

Asparagine, malic acid and mixed plasticized samples had a similar morphology as compared to S.D (chapter 3). The amount of plasticizer did not noticeably influence the particle morphology. Furthermore, based on the SEM micrographs no phase separation occurred as the particle surface appeared to be homogeneous.

Co-plasticized formulations (Figure 2c) exhibited a similar appearance as shown by all other formulated ones except isoleucine. Combining malic acid with glycerol, urea, or maltodextrin did not lead to visible phase separation, since particles exhibited a homogeneous surface.
Isoleucine plasticized samples manifested a different morphology (Figure 3(a, b, c)). The differences were more pronounced for increasing isoleucine concentrations with broken hollow particles observed for 30I.PS. The formation of hollow and broken particles may be explained by the “hydrophobic” nature of isoleucine. Compared to asparagine’s and malic acid, isoleucine has a relatively low HLB-index and is expected to be incompatible with the moisture-rich starch droplet during the drying step. As a result isoleucine will be transported to the outside of the particle where it rapidly forms a crystalline barrier for the evaporating moisture [26-28].

![SEM micrographs of (a) 20%A.P.S.D, 30%A.P.S.D and 40% A.P.S.D, (b) 20MA.PS, 30MA.PS and 40MA.PS (from left to right), (c) MA.G.PS and (d) MA.U.PS.](image)

**Figure 2** SEM micrographs of (a) pure isoleucine, (b) 20I.PS and (c) 30I.PS.

**Figure 3** SEM micrographs of (a) 20%A.P.S.D, 30%A.P.S.D and 40% A.P.S.D, (b) 20MA.PS, 30MA.PS and 40MA.PS (from left to right), (c) MA.G.PS and (d) MA.U.PS.
Crystallinity of freshly prepared TPS powder and films

The X-ray diffraction patterns of TPS powders and films processed with different plasticizers are shown in Figure 4. Analysis showed that all spray dried powders, except 20I.PS and 30I.PS, were amorphous (Figure 4a). Results are in line with previous chapters 3&4. The B- and V-type crystalline peaks at 2θ=17.2°, 19.6°, 22.3° and 23.6° in U.S were completely absent after solution spray drying (Figure 4a) [29, 30]. Short drying times prevented the association of starch chains into crystal lattices (chapter 2).

20I.PS and 30I.PS powders showed crystalline peaks (Figure 4a). These peaks were assigned to isoleucine crystalline domains present in the samples. Pure isoleucine showed XRD peaks at 2θ= 6.3°, 12.6°, 25.4°, 31.9° and 38.6°. In the isoleucine plasticized samples these peaks were also visible in the diffractograms. Furthermore, due to an increased isoleucine concentration, 30I.PS showed more intense crystalline peaks as compared to 20I.PS. Isoleucine solubility in aqueous solution is 4.1 [g/100g] at 25°C [31]. In 30I.PS the degree of super saturation was higher leading to increased crystal formation. Additionally, XRD analysis supported the observations of isoleucine crystals in SEM analyses.

![XRD spectra of (a) Freshly spray dried powder, (b) freshly prepared films by compression molding.](image)
XRD analysis of TPS films demonstrated that asparagine, malic acid and co-plasticized samples were amorphous (Figure 4b). By comparing Figure 4a and 4b it is clear that compression molding did not affect the amorphous character of the samples. The crystallinity was also not affected by combining malic acid with other used co-plasticizers. 20.I.PS and 30.I.PS samples again showed crystalline peaks, attributed to isoleucine (Figure 4b).

Freshly prepared malic acid plasticized and co-plasticized samples were transparent, irrespective of the amount of malic acid used. Asparagine plasticized samples were slightly hazy. Isoleucine samples were non-transparent, attributed to isoleucine crystals. Freshly prepared co-plasticized films were transparent. XRD analysis showed that all films lack B-type crystallinity except isoleucine plasticized films (Figure 4b) where an increase in isoleucine quantity, increased the amount of crystallinity.

**FT-IR analyses**

FT-IR analyses were performed to gain increased insight into the interactions between the used additives and starch (Table 3). As described in the previous chapters 3&4, a red-shift will occur for starch stretching vibrations of O-H, C-O and C=O upon hydrogen bond interactions. Bond elongation occurs due to hydrogen bond interactions, leading to lower stretching vibrations [32, 33].

Characteristic absorption bands of the TPS samples are depicted in Table 2. Absorption of starch occurred similarly as reported in previous chapters 3&4. The broad band at 3334 cm\(^{-1}\) was assigned to O-H stretching [9, 14, 34]. C-H stretching was assigned to the peaks at 2919-2915 cm\(^{-1}\) [9]. Absorption at 1150-1149 cm\(^{-1}\) and 1078 cm\(^{-1}\) was attributed to C-O stretching in C-O-H [13, 15]. Finally, bands at 1019-1018 cm\(^{-1}\) were allocated to C-O stretching from C-O-C [13, 15, 35].

The absorption band at 1019 cm\(^{-1}\) was clearly visible for all plasticized samples, indicating low crystallinity [36]. All spectra showed shifts in the fingerprint region of starch, indicating interaction between starch and additives. Comparing the spectra of asparagine and malic acid plasticized samples a red shift was visible upon increasing plasticizer levels. Hence, increasing plasticizer content gave rise to increased hydrogen bonding interactions.

In contrast, increasing isoleucine concentrations gave rise to a decreased amount of interactions between additive and starch molecules, as wavenumbers showed a blue shift (not in all cases) upon increased isoleucine contents. This was attributed to the hydrophobic nature of isoleucine giving rise to fewer interactions with starch.

Malic acid and mixed plasticized samples showed carbonyl absorption bands at 1711-1722 cm\(^{-1}\) (Table 3). Peaks in the range 1721-1750 cm\(^{-1}\) have been reported for esterification of starch and cellulose samples [37-43]. Although absorption signals near 1720 cm\(^{-1}\) may also be ascribed to carboxylic acid groups in di-acidic molecules present in the sample [6]. Increased absorption in the range 1200-1300 cm\(^{-1}\) was attributed to C-O stretching in esters [37, 40]. Peaks at 3334, 1659 and 1626 cm\(^{-1}\) in MA.U.PS were assigned to amide functionalities from urea. Absorption at 3334 cm\(^{-1}\) was attributed to N-H stretch, obscuring the O-H stretch band [9, 40]. The peak at 1659 cm\(^{-1}\) was assigned to C=O stretch from amide functionality. It also contains N-H bending vibrations. The band at 1626 cm\(^{-1}\) was assigned to N-H deformation [9, 40, 44]. The formation of ester linkages between starch and malic acid will be discussed later (*vide infra*).
Table 3
FT-IR absorption bands of the studied samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Wavelength [cm⁻¹]</th>
<th>3310-3270</th>
<th>2922-2921</th>
<th>1722-1711</th>
<th>1673-1614</th>
<th>1361</th>
<th>1019-1017</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S</td>
<td></td>
<td>3262</td>
<td>2922</td>
<td>--</td>
<td>--</td>
<td>1339</td>
<td>996</td>
</tr>
<tr>
<td>S.D</td>
<td></td>
<td>3310</td>
<td>2922</td>
<td>--</td>
<td>1635</td>
<td>1361</td>
<td>1018</td>
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<tr>
<td>20I.PS</td>
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<td>3239</td>
<td>2925</td>
<td>--</td>
<td>1615</td>
<td>1334</td>
<td>1020</td>
</tr>
<tr>
<td>30I.PS</td>
<td></td>
<td>3259</td>
<td>2922</td>
<td>--</td>
<td>1615</td>
<td>1330</td>
<td>1021</td>
</tr>
<tr>
<td>20A.PS</td>
<td></td>
<td>3283</td>
<td>2920</td>
<td>--</td>
<td>1622</td>
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<td>1019</td>
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<tr>
<td>30A.PS</td>
<td></td>
<td>3238</td>
<td>2923</td>
<td>--</td>
<td>1669,1616</td>
<td>1398</td>
<td>1019</td>
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<tr>
<td>40A.PS</td>
<td></td>
<td>3197</td>
<td>2926</td>
<td>--</td>
<td>1670,1622</td>
<td>1399</td>
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<tr>
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</tr>
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<td>1718</td>
<td>--</td>
<td>1341</td>
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</tbody>
</table>

Thermal Properties

Thermal properties of the TPS powders were investigated by TGA and DTGA (Figure 5 and Table 4). The threshold decomposition temperature (defined as the onset of degradation; see for example 294 °C for S.D in Table 4) indicates the highest processing temperature that can be used. The analysis of TGA data is however not always straightforward and the results cannot be directly converted into absolute features of the material under study [34]. However different sections can be allocated based on DTGA minima. For S.D, similar results were obtained as used in previous chapters 3&4.

Thermal analyses showed that the weight loss profile for all samples was similar (Figure 5). First loss of water and other volatiles occurred up to 110°C (section a). Subsequently, decomposition due to the presence of plasticizer is observed (section b₁), while the main degradation zone for starch is situated in section b₂. Section c indicates the propagation zone, leading to carbonaceous residues [17, 45]. The addition of plasticizer molecules caused a decrease in the decomposition temperature and an extra peak in the DTGA curve appeared (section b₁, Figure 5). For malic acid this decomposition temperature $T_{\text{dec}}$ decreased with an increase in the malic acid quantity which suggests more homogeneous blending and interaction of starch and malic acid or even covalent chemical interaction (*vide infra*).
Figure 5 TGA and DTGA-curves of solution spray dried amorphous powder samples.
Co-plasticized formulations depend on the type of plasticizer used. MA.U.PS showed the lowest $T_{\text{dec}}$ and MA.MD.PS the highest, i.e. 156°C and 260°C, respectively. This clearly proves the different nature of urea as plasticizer and the variation in interaction as a function of the chemical structure of the additive. The poor plasticization characteristic of maltodextrin was already discussed in a previous chapter.

The largest fraction of the starch-plasticizer formulations decomposes at the temperature defined as $T_{\text{dec,b2}}$ (chapter 3). It was found that the addition of plasticizer decreased this temperature, but did not give any clear relation with the quantity of plasticizer except for malic acid plasticized samples, i.e. with an increase in the quantity of malic acid the $T_{\text{dec,b2}}$ decreased. This again shows that the interaction of malic acid and starch differs from the interaction of starch and the other additives.

The results discussed so far lead to the conclusion that malic acid differs from classic plasticizers such as urea, formamide or glycerol. The acid seems to blend more homogenously with the starch matrix and forms molecular blends with specific thermal degradation characteristics. Similar effects have been shown for citric acid [12, 42] and it recently has been claimed that malic acid treated starch granules are non-swelling because of the formation of cross-links between the two components [46]. To better understand the role of malic acid in the studied formulations, the acid has also been submitted to the TGA and DTGA analysis. The analysis clearly showed that malic acid started to decompose at 160-170 °C with a peak decomposition temperature at 235°C. Comparing these data with the results shown in Figure 5, clearly suggests that malic acid and starch form a separate phase or even new chemical compositions during the mixing and drying operation.

**Differential scanning calorimetry**

DCS measurements on the samples showed that asparagine indeed acts as a plasticizer for starch as it reduces the glass transition temperature. Asparagine contains evenly distributed polar functional groups that interact with starch. Isoleucine tends to phase separate from starch (vide supra) and malic acid apparently homogenizes with starch without leading to an identifiable glass transition point.

The homogenisation effect observed for formulations containing malic acid is also found for the systems with mixtures of malic acid and urea, glycerol or maltodextrin. Although glass transition temperatures might be expected for urea and glycerol formulations, none of the samples showed a glass transition point. The typical b1 decomposition temperatures nevertheless did appear for the secondary additives (See Figure 5). Because malic acid in these formulations again leads to a reduction in the b2 decomposition temperature (i.e. homogenises with the starch matrix) and the secondary b1 decomposition signals do exist, it must be concluded that the contents of urea and glycerol were too low to register a true plasticizer effect [47].

**Retrogradation and moisture uptake**

Film retrogradation, measured by X-ray diffraction, was studied over a period of 56 days at various humidity conditions i.e. RH0%, 50% and 100% (Figure 6). The U.S line corresponds to the degree of crystallinity present in the reference feedstock material. As water influences the rearrangement of amorphous starch chains [3, 4], relative weight changes during storage were also recorded. All films showed an increase in the final crystallinity levels and relative weights in the
order RH100% > RH50% > RH0% (Figure 6). The most important changes in crystallinity and moisture absorption are found at RH100% although also some peculiar observations made at RH0% and RH50% will be discussed.

At RH0% all films maintained similar crystallinity levels and remained brittle (Figure 6) due to the absence of water. Even a small weight decrease was found for all films, attributed to film moisture loss at RH0% to the desiccant. The plasticizer content showed hardly any influence on recrystallization over time. Of all the formulations, 30LPS showed the highest crystallinity which may be attributed to the crystallinity of isoleucine itself (see X-ray diffraction section).

At 50% relative humidity the films increased in weight over time, and finally reached an equilibrium plateau. However, this increase in moisture absorption has little effect on the extent of retrogradation. Asparagine plasticized samples exhibited an increase in degree of crystallinity over time, attributed mainly to asparagine crystals. With increasing asparagine quantities the degree of crystallinity increased. Diffraction peaks of asparagine and isoleucine were in the area 2θ= 12°-31° (Figure 4). Consequently, the overall degree of crystallinity in isoleucine and asparagine plasticized samples was influenced by isoleucine and asparagine crystallization, respectively. Isoleucine and asparagine plasticized samples remained brittle upon storage. Malic acid and mixed plasticized samples showed no significant increase in crystallinity at RH50% over time.

![Figure 6 Degree of crystallinity of films after 56 days of storage at different humidity levels.](image)

Malic acid and mixed plasticized samples showed little retrogradation and the films remained flexible. Again low moisture uptake after 56 days at RH50% limited chain movement and crystallization (chapter 3&4). Malic acid plasticized or co-plasticized films stored at RH50% for 56 days led to a weight increase of only 10% to 13%. Hence, no notable differences were found upon addition of glycerol, urea, or maltodextrin combined with malic acid. Films stored at 50% humidity became flexible due to some moisture absorption and water acting as a plasticizer. However 20MA.PS was still brittle after 56 days, which could be because of the low quantity of malic acid present.
Malic acid as plasticizer for TPS starch?

Figure 7 Water sorption performance at 100% RH for 30% plasticizer contents fitted using Peleg model (X7 and X56 are the degree of crystallinity at day 7 and 56, respectively).

The moisture absorption data recorded at 100%RH are very indicative for the special behaviour of malic acid in the studied formulations (vide supra TGA analysis). Figure 7 presents an overview of the data for the 30%w single formulations including the crystallinity data at day 7 and day 56 (including SD as a reference). The data for SD, isoleucine and asparagine could all be fitted to the Peleg absorption model. The 30MA data however only followed the Peleg fit for the initial moisture absorption of two weeks; after that period a more complex fitting model had to be applied. The reason that the Peleg curve fit no longer applies is probably due to the fact that moisture absorption no longer approaches the equilibrium state determined by the otherwise crystalline structure of a starch matrix. Even in the formulation containing 30Urea (See previous chapter 4), it has been shown that after a certain period of moisture absorption, retrogradation expels water (and possibly urea) to lead to an equilibrium situation. The fact that the malic acid formulation does not lead to equilibrium is probably due to very strong malic acid starch interaction or possibly irreversible grafting of malic onto the starch backbone. Further evidence for the peculiar behaviour of malic acid is shown in Figures 8 and 9 in which the data for malic acid at different concentrations and for plasticizer combinations are fitted. Apart from the maltodextrin reference sample in Figure 9, all data had to be fitted using a modification of the Peleg model.
Figure 8 illustrates the results of duplicate experiments with malic acid concentrations. All cases indicate little retrogradation and, as expected, high moisture absorption. It is remarkable though to note that the absorption/MA-concentration relationship seems to contain a maximum because there is an increase in absorption from 20%MA to 30%MA while the curve for 40%MA is lower than the one for 30%MA. The explanation for this phenomenon must be found in the specific interaction of malic acid and starch. Recent literature [16, 46] suggests that malic acid is, like citric acid, capable of covalent bonding onto starch or cross-linking through the formation of intermediate intra-molecular anhydrides. If that indeed is the case, the 40%MA sample might be considered to be more cross-linked and therefore less capable to absorb moisture. FT-IR analysis of the samples (as discussed before) was not conclusive about this proposition; TGA data mainly suggested some strong interactions between the components of the formulation. Further experimentation to prove the suggested cross-linking is currently on-going.

Figure 9 provides further evidence for the strong malic acid – starch interaction. The moisture absorption and the retrogradation data shown again point to the dominant interaction of malic acid. Compared to earlier work with glycerol, urea and maltodextrin (Chapter 2 and 3) the retrogradation of the current formulations seems to be inhibited by the presence of malic acid.

Storing the films at RH100% (Figure 7) indeed created significant differences in appearance because of excessive moisture uptake for some of the samples. Asparagine plasticized samples became soft; whereas isoleucine plasticized samples remained brittle. Isoleucine actually exuded from the matrix and after 14 days isoleucine crystals were visible on the surface of the films. As a result, 30I.PS films completely disintegrated after 56 days of storage at RH100%, which can be attributed to super saturation of isoleucine and the recrystallization of isoleucine and starch (Figure 10).
The degree of crystallinity of asparagine plasticized TPS films increased with asparagine concentration hence, asparagine crystallization contributed to a higher overall degree of crystallinity of the formulation. Malic acid and co-plasticized films remained flexible and maintained structural integrity during the 56 day test period. MA containing films displayed the largest relative weight increase; the highest for the urea containing combination and the lowest for MA.MD.PS. This large weight increase can be attributed to their amorphous character [48]. Furthermore, malic acid prevented extensive retrogradation of starch for at least 56 days at RH100%. Even the addition of 15% of malic acid in the 15:15 glycerol and urea mixtures was sufficient to postpone starch retrogradation for 56 days. On the other hand, MA.MD.PS plasticized samples showed retrogradation at 100%RH. This was attributed to poor plasticizer quality of maltodextrin (chapter 3). Malic acid was thought to have the most effect on restricting retrogradation, since malic acid plasticized samples (30MA.PS) extended retrogradation to a greater extend as compared to 30% glycerol, urea, or maltrodextrin as discussed in previous chapters 3&4. Strong hydrogen bonding between starch and malic acid and possibly some cross-linking gave rise to limited retrogradation.

The degree of crystallinity of the co-plasticized samples was comparable to 30MA.PS except for the maltodextrin formulation (Figure 6). Moisture sorption for MA.G.PS and MA.U.PS was extensive and contributed to large portions of the films remaining amorphous [48]. MA.U.PS showed a higher increase in relative weight as compared to MA.G.PS. MA.MD.PS showed lowest relative weight and highest degree of crystallinity as compared to all malic acid formulations. This was attributed to the incorporation of urea which showed a higher relative weight increase as compared to glycerol at RH100% (chapter 4). Furthermore, MA.U.PS exhibited more hydrophilic end-groups because of more urea molecules being present as compared to glycerol and maltodextrin. These polar groups at the end of the molecule are also expected to be more accessible to water and therefore increase moisture uptake. MA.U.PS showed gelification after 14 days. This indicated that malic acid increased the structural integrity by reacting with starch. According to a
previous chapter 4, 30U.PS showed gelification already after 3 days at RH100% and 131% weight increase.

Suppression of retrogradation in the malic acid plasticized and co-plasticized samples was attributed to partial esterification and cross-linking. The un-reacted malic acid acted as a plasticizer, while the groups introduced on the starch chains acted as defects in the crystal structures of starch.

Malic acid shows structural resemblance with citric acid. Citric acid is proven to be a crystallization inhibitor for starch, because of esterification and strong hydrogen bonding interactions with starch [12, 43]. Different plasticizer amounts of malic acid displayed no significant differences in retrogradation after 56 days at RH100%. Research of Yu et al. (2005) showed that citric acid quantities of 3 weight% were enough for suppressing starch re-crystallization for 70 days at 100% relative humidity [12]. Hence, it is plausible that 20% of malic acid was more than enough for preventing retrogradation within 56 days at RH100%.
Table 4. Degree of crystallinity, relative weight, thermal properties and mechanical properties of TPS films.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture contents Powder [%]</th>
<th>Thermal properties [°C]</th>
<th>Mechanical properties</th>
<th>Degree of crystallinity of films</th>
<th>Relative weight change films</th>
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<td></td>
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<td>291</td>
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*a S.D could not be tested due to brittleness of the test sample.
Mechanical Properties

One of the most important parameters to evaluate polymer films is mechanical strength, especially for those used as packaging materials [5]. The mechanical behaviour of 30% plasticized and co-plasticized TPS films were assessed after equilibration in 100%RH for 1 day (Figure 11). Due to the brittle nature, starch S.D could not be tested.

Flexibility of the created TPS films increased in the order isoleucine < asparagine < malic < MA.MD.PS < MA.G.PS < MA.U.PS. Increasing hydrogen bond capabilities per molecule reduced crystallinity and tensile strength, but increased strain. More hydrogen bonding functionalities increased the capabilities of the plasticizer to disrupt starch inter- and intra-molecular hydrogen bonds and to absorb moisture. Hereby starch chain mobility and thus flexibility was improved. Isoleucine plasticized samples were relatively strong but brittle due to increased film crystallinity.

![Figure 11](image.png)

Figure 11 Mechanical properties of the TPS films.

The results of co-plasticized formulations were comparable; they showed high elongation at break, low tensile strength and low moduli. It indicated that malic acid influenced esterification and urea, glycerol, and maltodextrin interfered with the remaining starch-starch hydrogen bonds. Hence, strain was limited by starch cross-links. Un-reacted plasticizer molecules act as plasticizers, increasing strain to a maximum of 160% for MA.G.PS. The strain at break increased when compared to our previous chapter 4: 30G.PS, 30U.PS and 30MA.PS. This was attributed to glycerol and urea being effective plasticizers for starch, disturbing starch inter- and intra-molecular hydrogen bonds. The MA.MD.PS co-plasticized sample showed lower elongation but same strength compared to urea and glycerol co-plasticized samples. This could be due to crystallinity and poor plasticizer nature of maltodextrin itself. In a previous chapter 3, malto-oligosaccharides of different molecular weights, i.e. dextrose equivalents (DE) were studied to investigate the effect as plasticizer or processing aid. The study proved the poor plasticizer characteristics of maltodextrin with poor
interaction between starch and maltodextrin. The samples showed no $T_g$ and high rate of retrogradation.

In addition, malic acid plasticized samples showed increased structural integrity upon storage explaining the increased strain. Disproportionate changes in tensile properties of citric-acid and glycerol co-plasticized starch films is reported in another research [12], similar to results reported in the present study. It was thought that, in this study, the co-plasticizer (glycerol, urea, and maltodextrin) effectively disrupts hydrogen bond interactions between starches, whereas malic acid gives rise to partial esterification/cross linking of starch, thus limiting strain to around 135%.

**Conclusion**

The effect of some selected plasticizers i.e. isoleucine, asparagine, malic acid and combinations of malic acid with glycerol, urea, and maltodextrin on the retrogradation of amorphous TPS films was studied. Plasticizers with similar molecular weights but different hydrogen bond forming units were chosen to investigate the ability to inhibit retrogradation of starch.

Freshly prepared powder and film samples of asparagine and all malic acid (including co-plasticized) plasticized starch formulations were amorphous. All malic acid containing films were transparent, whereas asparagine plasticized films were slightly mat. Isoleucine plasticized starch powder and films exhibited crystal structures attributed to isoleucine and were non-transparent.

Retrogradation was suppressed for the malic acid and co-plasticized samples for 56 days at all relative humidity levels. No significant difference in retrogradation was observed for any of the formulations except the MA.MD.PS sample. It showed maximum retrogradation i.e. 12% , whereas others remained below 7.5%. Apparently 15% w of malic acid in the formulation is sufficient to suppress retrogradation at RH100% for 56 days. Grafting and or cross- linking and strong hydrogen bonding between starch and malic acid is considered to prevent the normally observed behaviour of starch plasticizer mixtures.

Isoleucine showed poor interactions with starch and segregated during spray drying, limiting the plasticizing effect of isoleucine. 30LPS films stored at RH100% kept their structural integrity for only 28 days, whereas all other samples maintained their structure for at-least 56 days. Asparagine plasticized samples showed retrogradation at 50% and 100% relative humidity. In terms of thermal and mechanical performance, the tested additives seem to follow the heuristics known for starch plasticizers. No $T_g$ is recorded for any of the plasticizers except for the asparagine plasticized formulation. All films are stable up to 160 °C, crystallinity and retrogradation behaviour is correlated with strength and strain is related to the extent of moisture absorption.

**References**


Chapter 5
