Influence of plasticizer with different functional groups on thermoplastic starch

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ABSTRACT: Retrogradation of amorphous thermoplastic starch (TPS) films obtained by compression molding of spray dried modified potato starch was investigated. The aim was to investigate the influence of plasticizers with similar molecular weight but different functionality, i.e., isoleucine, asparagine and malic acid, on the properties of plasticized 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 as amorphous powders and films. Malic acid was identified as a strong antiretrogradation agent as it inhibited recrystallization of starch over the full range of humidity levels. Malic acid was also found to inhibit the retrogradation of formulations containing urea, glycerol and maltodextrin. The converse of the strong inhibition implied strong moisture absorption and high strain at break values, and low tensile strengths. Malic acid was also identified as a potential crosslinking agent to control swelling of starch-based products. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42012.

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. Upon retrogradation, fractions of the polymeric starch chains rearrange and form crystal-line domains,[1,2] which alter the physical and mechanical properties of the material and reduce the number of practical applications. Similar to synthetic polymers, the increase in crystallinity leads to an increased modulus, brittleness, and a decrease in strain. On the other hand, the absence of retrogradation may lead to uncontrolled moisture absorption and loss of mechanical properties. Therefore, reducing the dynamic behavior 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.[4–8]

Several studies focussed on the effect of different plasticizers on TPS films.[9] Polyols, such as glycerol, xylitol, and sorbitol closely resemble the starch structure and are therefore widely used.[7,10–12] Likewise, citric acid was shown to act as plasticizer and also inhibited recrystallization of starch.[13] 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 behavior 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.[14–16] The effectiveness of amide structures and citric acid in suppressing the retrogradation has been attributed to efficient hydrogen bonding or chemical reaction.[17] In addition, literature clearly showed a higher effectiveness for low-molecular-weight plasticizers in suppressing retrogradation.[11,14,15]

Previous studies showed that dissolving starch and plasticizer together in an aqueous solution followed by drying and film production resulted in film properties different from films produced by classic extrusion technology or solvent casting.[8,18] 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
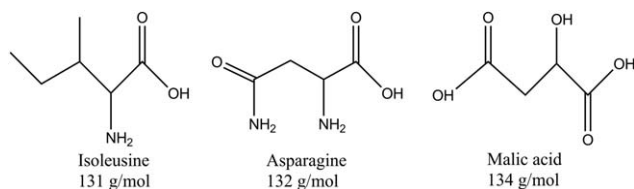
Figure 1. Natural plasticizers, having similar molecular weights.

plasticizer or processing aid. It was observed that in the dry starch formulation made by the spray-drying process, the additives did not act as plasticizer. The rate of moisture uptake appeared to be the highest for the lowest molecular weight additive with an opposite trend for the equilibrium level of recrystallization. In the next work, abundantly used plasticizers, i.e., glycerol and urea, were used separately and in combinations with maltodextrin. It was found that glycerol and urea acted as effective plasticizers, i.e., lowered the T_g of the system, but that the retrogradation behavior differed significantly. Upon exposure to high humidity conditions, glycerol stimulated rapid retrogradation while urea delayed this process but promoted moisture absorption.

Against this backdrop a study was undertaken with the aim to generate better understanding about the interaction of spray dried starch with low molecular weight and hydrogen bonding additives. 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.

In the second part of the study combinations of malic acid and the more traditional additives 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. Particle morphology, thermal properties and molecular interactions between the powder components were tested by scanning electron microscopy (SEM), thermal gravimetric analysis (TGA) and differential TGA (DTGA), and Fourier transform infrared spectroscopy (FT-IR) analysis, respectively. For the films retrogradation, moisture uptake and mechanical properties were evaluated.

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 ($\geq$98%), and DL-malic acid (99%) were purchased from Sigma-Aldrich (the Netherlands). Maltodextrin (Dextrose equivalent = 19.1) was kindly supplied by Cargill (France). Codes for the starch formulations are depicted in Table I. 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 min applying a stirring rate of 600 RPM to prepare homogeneous solutions. The obtained solutions were fed to a Buchi mini spray drier B-191(Switzerland) equipped with a 0.7-mm nozzle. The procedure to produce amorphous powder was adopted as described in previous work.[8,19]

Preparation of TPS-Films
TPS films (diameter of films are Ø10 mm $\times$ 0.5 mm) were produced by compression molding the TPS powder, using a Fontijne Holland Table Press TH400. Samples were compressed at 2.5×10^6 Pascal for 5 min. The molding temperature for all samples was 140°C.[18]

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

X-ray Diffraction
The crystalline structure of the freshly produced spray dried powders and films were elucidated using X-ray diffraction (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.

Freshly produced powder was analyzed using a sample holder with a sample compartment of diameter Ø25 mm $\times$ 2.0 mm.

Table I. Code Names, Corresponding Description, and Plasticizer Molecular Weight (MW)

Code	Description (w/w of starch)	MW (g mol^{-1})
U.S	Feedstock material	–
S.D	Spray dried starch	–
20I.PS	20% isoleucine plasticized starch	131
30I.PS	30% isoleucine plasticized starch	131
20A.PS	20% asparagine plasticized starch	132
30A.PS	30% asparagine plasticized starch	132
40A.PS	40% asparagine plasticized starch	132
20MA.PS	20% malic acid plasticized starch	134
30MA.PS	30% malic acid plasticized starch	134
40MA.PS	40% malic acid plasticized starch	134
MA.G.PS	15% malic acid, 15% glycerol plasticized starch	–
MA.U.PS	15% malic acid, 15% urea plasticized starch	–
MA.MD.PS	15% malic acid, 15% maltodextrin plasticized starch	–

Table II. Constant Values (k_1, k_2, c_0, c_1, and c_2) and Regression Coefficient (R^2) for Sorption Curve eqs. ((2) and (3); Peleg Model) of TPS Films at 100RH

Sample	M_0	k_1	k_2	c_0	c_1	c_2	R^2
S.D	5.2	4.2×10^{-2}	1.5×10^{-2}	–	–	–	0.99
30I.PS	2.5	3.4×10^{-2}	1.8×10^{-2}	–	–	–	0.99
30A.PS	2.4	4.1×10^{-2}	1.1×10^{-2}	–	–	–	0.99
20MA.PS	1.3	–	–	0.017	1.2×10^{-2}	-9.2×10^{-5}	–
30MA.PS	1	–	–	0.026	6.3×10^{-3}	-6.4×10^{-5}	–
40MA.PS	0.9	–	–	0.017	8.2×10^{-3}	-6.2×10^{-5}	–
(15 : 15)MA.G.PS	0.5	–	–	0.012	5.4×10^{-3}	-5.5×10^{-5}	–
(15 : 15)MA.U.PS	0.5	–	–	0.011	5×10^{-3}	-6.4×10^{-5}	–
(15 : 15)MA.MD.PS	0.8	–	–	9.5×10^{-3}	8.9×10^{-3}	-1×10^{-4}	–
30MD.PS	2	2.8×10^{-2}	1.4×10^{-2}	–	–	–	0.99

Ø10 mm × 0.5 mm freshly produced TPS films were analyzed using an adjustable sample holder.

Thermal Gravimetric Analysis

Open pan TGA of freshly produced TPS powder was performed in a Perkin Elmer TGA 7.5–10 mg. Similar procedure was applied to study the weight change as a function of temperature as described in previous work.[8] The decomposition temperature (T_{dec}) was determined using the method described in literature.[20]

Differential Scanning Calorimetry

The glass transition temperature (T_g) of the freshly produced TPS powder samples was determined by differential scanning calorimetry (DSC) measurements, using a DSC-60 Shimadzu Co. Prior to analysis a reference was constructed using an empty aluminum pan. Samples were scanned twice at a rate of $10°C \ min^{-1}$ from $10°C$ to $200°C$. Open pan measurement was performed in order to remove any remaining water from all samples in the first run and measurements were taken during the second run.

Fourier Transform Infrared Spectroscopy

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 × 0.5 mm) was measured under different relative humidity conditions: 0%RH (dried silica), 50%RH (35.64%$CaCl_2$ 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. The degree of crystallinity was determined using the method described in literature.[21] Similar procedure was used for integration and

calculation of crystallinity as explained in previous studies.[8,18] The software program Origin 8.1 was used for integrating the peaks. The crystalline area was measured between 12° and 31°. The total area was the area under the curve from 12° to 40° in the XRD spectrum. 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 the following equation 1:[22]

$$\text{moisture uptake} = \frac{\text{weight}_{\text{day},n} - \text{weight}_{\text{day},0}}{\text{weight}_{\text{day},0}} \times 100\% \qquad (1)$$

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 [eq. (2)] except malic acid plasticized and coplasticized formulations.[23] Malic acid plasticized and coplasticized formulations were fitted using a modified Peleg model [eq. (3)].

$$M_t = M_0 + \left(\frac{t}{(k_1 + k_2 t)} \right) \qquad (2)$$

$$M_t = M_0 + \left(\frac{t}{(c_0 + c_1 t + c_2 t^2)} \right) \qquad (3)$$

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).[24] The Peleg parameters,[23] k_1 and k_2 are shown in Table II. 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.[24] The regression coefficient R^2 values were found to be very high in all cases ($R^2 > 0.99$). The Peleg model [eq. (2)] appeared not adequate for malic acid formulations. In those cases the polynomial expression [eq. (3)] is used to model the moisture absorption.

Mechanical Testing

The mechanical properties include the tensile strength (TS) and percentage elongation at break (%E). Five or more specimens
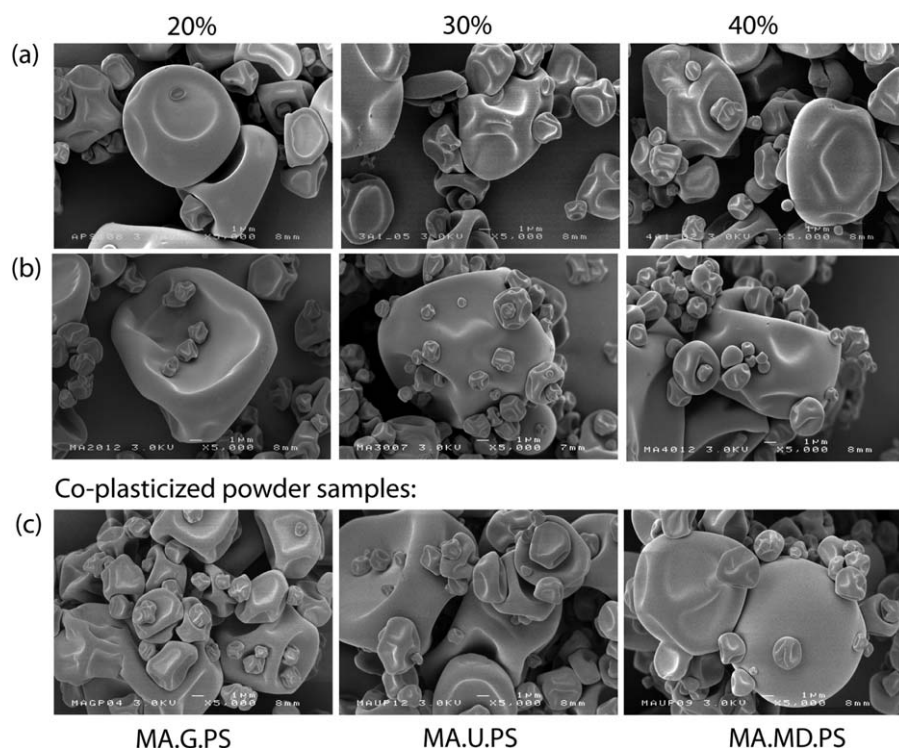
Figure 2. Scanning electron micrographs of (a) 20A.PS, 30A.P.S, and 40A.PS, (b) 20MA.PS, 30MA.PS, and 40MA.PS (from left to right), and (c) MA.G.PS, MA.U.PS, and MA.MD.PS.

were tested using an Instron 4301 tensile tester in accordance with ASTM D1708. The specimens used were of standard dumb bell shaped. The nominal dimensions of each specimen were approximately as following; width of narrow section was 13 mm, length of narrow section was 57 mm. The overall length and distance between grips of specimen was 165 mm and 115 mm, respectively. The crosshead speed used was 10 mm min^{-1}. The samples were conditioned at 25°C and 100% RH in humidity chamber for 24 h and the thickness and width of the samples were measured before testing.

RESULTS AND DISCUSSION

Controlling the retrogradation of TPS based material is a very interesting and important topic for the application of TPS and natural plasticizers. The selection of plasticizer was done on the bases of biodegradable having similar molecular weight but different functional groups.

Powder Morphology

Figure 2 shows the granular morphology of the spray dried powder samples. In line with literature,[8,18] 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 compared to feedstock (Figure 2). Morphology of powder granules was similar to previous results.[8,18] Particles were spherical in nature however they contained dents, which is typical for spray dried powders.[19,25,26] The degree of shrinkage is determined by the drying sequence.[27,28] This diffu-

sion leads to the formation of dents in the particle surface.[19] The deformation of the particles after spray drying also indicates the formation of hollow particles.[28] Asparagine, malic acid, and mixed plasticized samples had a similar morphology as compared to S.D.[8] The amount of plasticizer did not noticeably influence the particle morphology.

Coplasticized formulations [Figure 2(c)] exhibited a similar appearance as shown by all other formulated once 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–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 hydrophilic–lipophilic balance (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.[29–31]

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 4(a)]. Results are in line with previous work.[8,18] The B- and V-type crystalline peaks at $2\theta = 17.2°$,
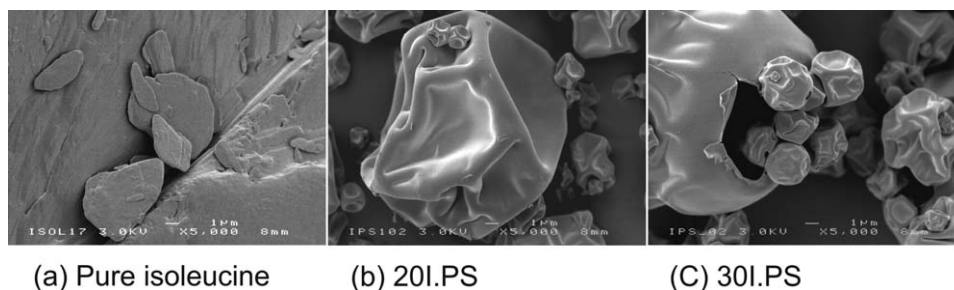
Figure 3. Scanning electron micrographs of (a) pure isoleucine, (b) 20I.PS, and (c) 30I.PS.

19.6°, 22.3°, and 23.6° in U.S were completely absent after solution spray drying [Figure 4(a)].[32,33] Short drying times prevented the association of starch chains into crystal lattices.[19]

20I.PS and 30I.PS powders showed crystalline peaks [Figure 4(a)]. These peaks were assigned to isoleucine crystalline domains present in the samples. Pure isoleucine showed XRD peaks at $2\theta = 6.3°$, 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/100 g) at 25°C.[34] 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 analysis of TPS films demonstrated that asparagine, malic acid, and coplasticized samples were amorphous [Figure 4(b)]. By comparing Figure 4(a,b) 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 coplasticers. 20I.PS and 30I.PS samples again showed crystalline peaks, attributed to isoleucine [Figure 4(b)].

Freshly prepared malic acid plasticized and coplasticized 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. All freshly prepared coplasticized films were transparent. XRD analysis showed that all films lack B-type crystallinity except isoleucine plasticized films [Figure 4(b)] 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 III). As described in the literature,[8,18] 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.[35,36] Characteristic absorption bands of the TPS samples are depicted in Table II. Absorption of starch occurred similarly as reported in previous work.[8,18] The broad band at 3334 cm^{-1}
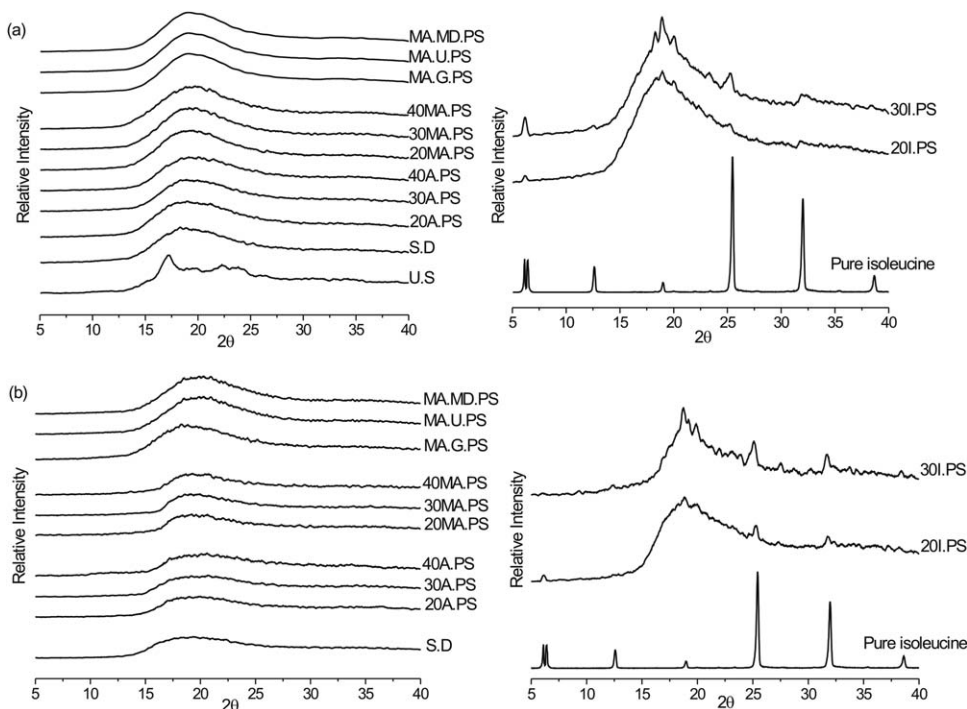
Figure 4. XRD spectra of (a) freshly spray dried powder and (b) freshly prepared films by compression molding.

Table III. FT-IR Absorption Bands of the Studied Samples

Samples	Wavelength (cm^{-1})					
	3310-3270 (O–H stretching)	2922-2921 (C–H stretching)	1722-1711 (presence of carbonyl group in films)	1673-1614 [C–O stretching (amide)]	1361 (C–O–H bending)	1019-1017 (C–O bond stretching)
U.S	3262	2922	–	–	1339	996
S.D.	3310	2922	–	1635	1361	1018
20I.PS	3239	2925	–	1615	1334	1020
30I.PS	3259	2922	–	1615	1330	1021
20A.PS	3283	2920	–	1622	1399	1019
30A.PS	3238	2923	–	1669, 1616	1398	1019
40A.PS	3197	2926	–	1670, 1622	1399	1019
20MA.PS	3294	2922	1722	–	1334	1019
30MA.PS	3278	2926	1714	–	1337	1019
40MA.PS	3279	2926	1713	–	1339	1019
MA.G.PS	3334	2919	1717	–	1340	1019
MA.U.PS	3334	2915	1713	1627	1359	1019
MA.MD.PS	3351	2822	1718	–	1341	1018

was assigned to O–H stretching.[10,15,37] C–H stretching was assigned to the peaks at 2919–2915 cm^{-1}.[10] Absorption at 1150–1149 cm^{-1} and 1078 cm^{-1} was attributed to C–O stretching in C–O–H.[14,16] Finally, bands at 1019–1018 cm^{-1} were allocated to C–O stretching from C–O–C.[14,16,38] The absorption band at 1019 cm^{-1} was clearly visible for all plasticized samples, indicating low crystallinity.[39]

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 III). Peaks in the range 1721–1750 cm^{-1} have been reported for esterification of starch and cellulose samples.[40–46] 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.[40,43]

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.[10,43] 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.[10,43,47] The formation of ester linkages between starch and malic acid will be discussed later (*vide infra*).

Thermal Properties (TGA and DTGA Analysis)

Thermal properties of the TPS powders were investigated by TGA and DTGA (Figure 5 and Table IV). The threshold decomposition temperature (defined as the onset of degradation; see for example 294°C for S.D in Table IV) indicates the highest processing temperature that can be used. The analysis of TGA data is however not always straight forward and the results cannot be directly converted into absolute features of the material under study.[37] However, different sections can be allocated based on DTGA minima. For S.D, similar results were obtained as used in previous work.[8,18] 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 b$_1$), while the main degradation zone for starch is situated in Section b$_2$. Section c indicates the propagation zone, leading to carbonaceous residues.[20,48] The addition of plasticizer molecules caused a decrease in the decomposition temperature and an extra peak in the DTGA curve appeared (Section b$_1$, Figure 5). For malic acid this decomposition temperature T_{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*). Coplasticized formulations depend on the type of plasticizer used. MA.U.PS showed the lowest T_{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 previous work.[8] The largest fraction of the starch–plasticizer formulations decomposes at the temperature defined as $T_{dec,b2}$.[8]

It was found that the addition of plasticizer decreased this temperature, but did not give any clear relation with the
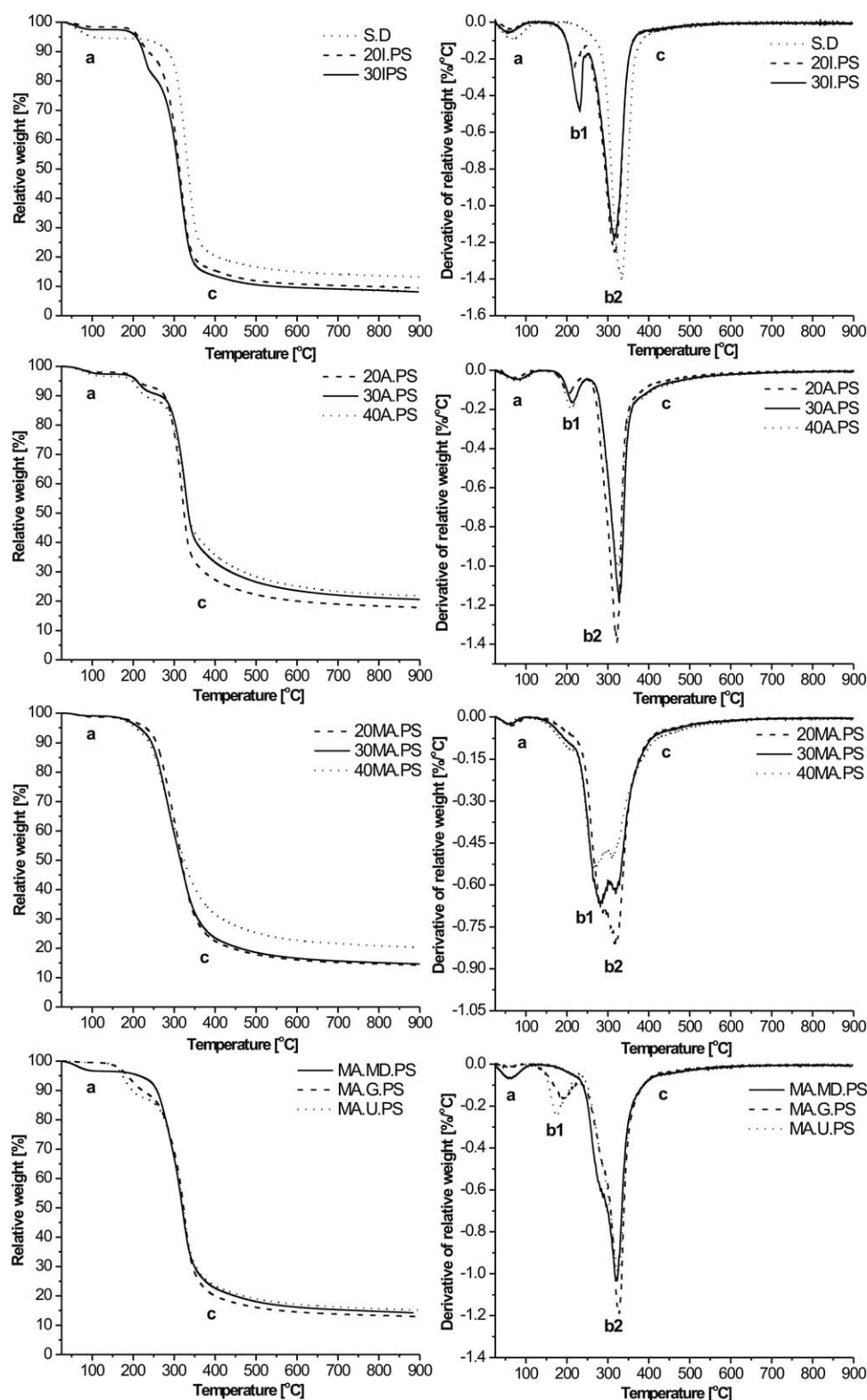
Figure 5. TGA and DTGA curves of solution spray dried amorphous powder samples.

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[13,45] and it

Table IV. Degree of Crystallinity, Relative Weight, Thermal Properties, and Mechanical Properties of TPS Films

Samples	Moisture contents powder (%)	Thermal properties (°C)				Mechanical properties			Degree of crystallinity of films			Relative weight change films	
										RH 100%		RH 100%	
		Onset, T_{dec}	Peak, $T_{dec,b1}$	$T_{dec,b2}$	T_g	Modulus (MPa)	Tensile strength (MPa)	Strain (%)	0 day (%)	7 day	56 days	1 day	56 days
S.D	5.2	294	–	334	No	a	a	a	0.5	17.9	18.7	29.1	64.6
20I.PS	1.6	199	217	317	No	–	–	–	5.5	–	–	18.1	65.3
30I.PS	2.5	203	231	316	No	1145 (48)	8.5 (4.8)	0.93 (0.6)	6.2	17.1	19.7	25.2	56.5
20A.PS	1.9	193	203	321	No	–	–	–	0.6	–	–	27.1	64.7
30A.PS	2.4	190	214	327	123	821 (210)	6.6 (1.9)	10.5 (8.9)	0.6	24.3	28.1	35.9	93.2
40A.PS	3.1	190	212	321	112	-	–	–	0.6	–	–	31.8	123.2
20MA.PS	1.3	255	287	317	no	–	–	–	0.8	–	–	48	328.3
30MA.PS	1.0	232	281	314	no	185 (42)	1.6 (0.2)	11.3 (5.7)	0.8	1.6	5.5	54.1	359.8
40MA.PS	0.9	226	273	310	no	–	–	–	0.8	–	–	50	361
MA.G.PS	0.5	162	194	327	no	1.9 (0.6)	0.07 (0.01)	160 (24)	0.7	3.8	4.3	66.8	388.0
MA.U.PS	0.5	156	175	324	no	1.9 (2.3)	0.09 (0.01)	155 (19)	0.6	3.3	7.3	69.1	618.9
MA.MD.PS	0.8	260	291	322	no	0.08 (0.04)	0.05 (0.02)	134.5 (15)	1.5	9.7	11.9	58.3	295.8

S.D could not be tested due to brittleness of the test sample.

recently has been claimed that malic acid treated starch granules are non-swelling because of the formation of crosslinks between the two components.[49]

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

DSC 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 homogenization effect observed for formulations containing malic acid is also found for the systems with mixtures of malic acid and urea, glycerol or maltodextrin (Figure 5). Although glass transition temperatures might be expected for urea and glycerol formulations, none of the samples showed a glass transition point. It must be concluded that the contents of urea and glycerol were too low to register a true plasticizer effect.[18,50]

### 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(a)]. The U.S line corresponds to the degree of crystallinity presented 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(a)]. 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(a)] 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, 30I.PS 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 (Figure 4). 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. 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.[8,18]
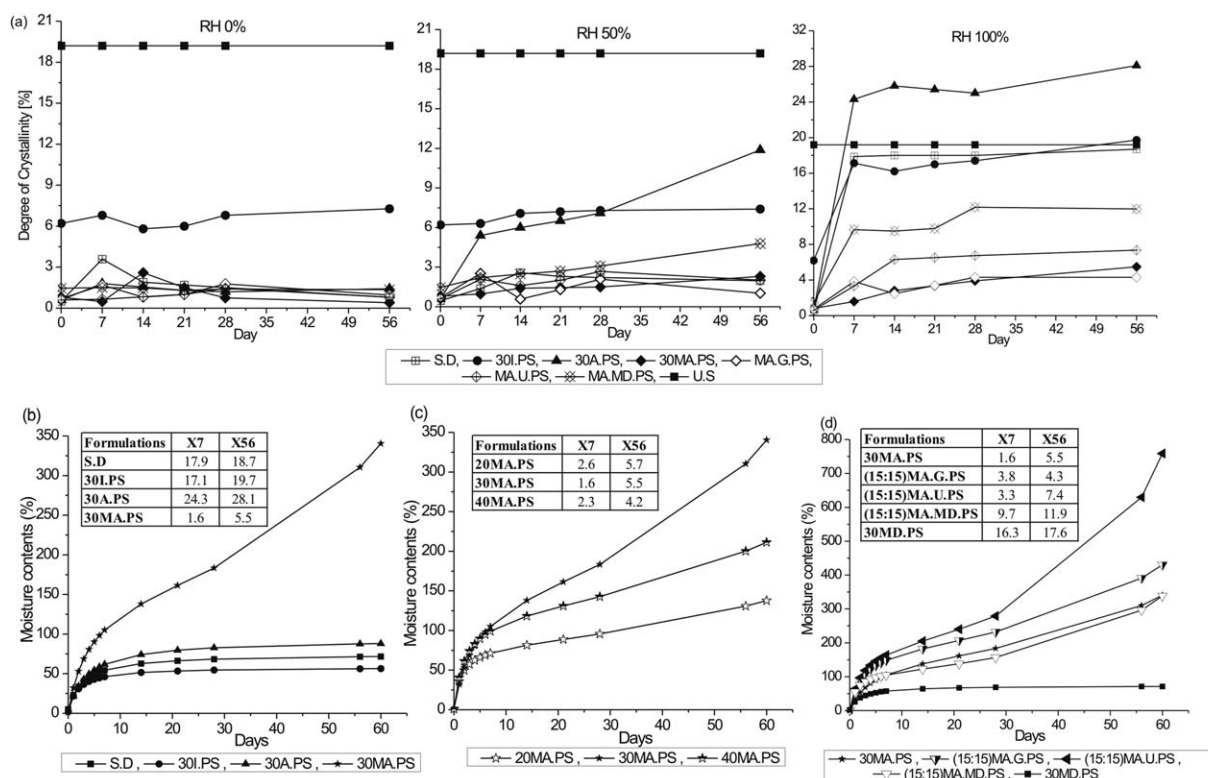
Figure 6. (a) Degree of crystallinity of films after 56 days of storage at different humidity levels, Water sorption performance at 100% RH fitted using Peleg model (b) for 30% plasticizer contents at 100% RH, (c) various malic acid plasticized formulations at 100% RH, (d) for malic acid plasticized and coplasticized formulations fitted at 100% RH (X7 and X56 are the degree of crystallinity at Days 7 and 56, respectively).
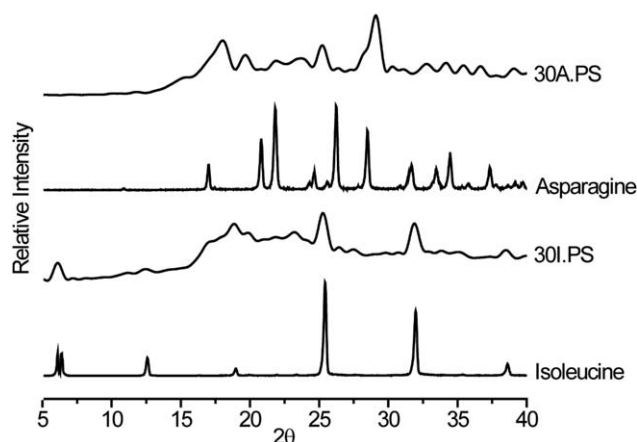
Malic acid plasticized or coplasticized films stored at RH50% for 56 days led to a weight increase of only 10–13%. Hence, no notable differences were found upon addition of glycerol, urea, or maltodextrin combined with malic acid. Films stored at 50% humidity became flexible due to some moisture absorption 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. The moisture absorption data recorded at 100%RH are very indicative for the special behavior of malic acid in the studied formulations (*vide supra* TGA analysis). Figure 6(b) presents an overview of the data for the 30%w single formulations including the crystallinity data at Days 7 and 56 (including SD as a reference). Moisture absorption for 30MA no longer approaches the equilibrium state determined by the crystalline structure of the starch matrix. Even in the formulation containing 30Urea,[18] 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 malic acid formulation does not lead to equilibrium was due to very strong malic acid starch interaction or possibly irreversible grafting of malic onto the starch backbone. Further evidence for the peculiar behavior of malic acid is shown in Figure 6(c,d). The data for malic acid at different concentrations and for plasticizer combinations are fitted. Apart from the maltodextrin reference sample in Figure 6(d), all data had to be fitted using a modification of the Peleg model.

Figure 6(c) 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. Recent literature[17,49] suggests that malic acid is, like citric acid, capable of covalent bonding onto starch or crosslinking through the formation of intermediate intra-molecular anhydrides. 40%MA sample considered to be more crosslinked and therefore less capable to absorb moisture. TGA data suggested strong interactions between the components of the formulation. Further experimentation to prove the suggested crosslinking is currently ongoing. Figure 6(d) provides further evidence for the strong malic acid–starch interaction. Compared to earlier work with glycerol, urea and maltodextrin, the retrogradation of current formulations inhibited by the presence of malic acid.[8,18]

Storing the films at RH100% [Figure 6(b)] 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 7).

The degree of crystallinity of asparagine plasticized TPS films increased with asparagine concentration hence, asparagine

Figure 7. X-ray patterns of pure isoleucine, pure asparagine and plasticized films at RH 100% on Day 56.

crystallization contributed to a higher overall degree of crystallinity of the formulation. Malic acid and coplasticized films remained flexible and maintained structural integrity during the 56 day test period. MA containing films displayed the largest relative weight increase; the MA.U.PS films showed the highest relative weight increase whereas MA.MD.PS showed the lowest. This large weight increase attributed to their amorphous character.[51]

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.[8] Malic acid was thought to have the most effect on restricting retrogradation. Strong hydrogen bonding between starch and malic acid and possibly some crosslinking gave rise to limited retrogradation.

The degree of crystallinity of the coplasticized samples was comparable to 30MA.PS except for the maltodextrin formulation [Figure 6(a)]. Moisture sorption for MA.G.PS and MA.U.PS was extensive and contributed to large portions of the films remaining amorphous.[18,51]

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%.[18] 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 increasing moisture uptake. MA.U.PS showed gelification after 14 days. This indicated that malic acid increased structural integrity by reacting with starch. According to previous results,[18] 30U.PS showed gelification already after 3 days at RH100% and 131% weight increase.

Suppression of retrogradation in the malic acid plasticized and coplasticized samples was attributed to partial esterification and cross linking. The unreacted 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.[13,46] Different plasticizer amounts of malic acid displayed no significant differences in retrogradation after 56 days at RH100%. Research of Yu et al.[13] showed that citric acid quantities of 3 wt % were enough for suppressing starch recrystallization for 70 days at 100% relative humidity. Hence, 20% of malic acid was more than enough for preventing retrogradation within 56 days at RH100%. Malic acid proved to be an excellent inhibitor of TPS retrogradation. However, the water adsorption in the presence of malic acid increases significantly under applied humidity conditions.

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 behavior of 30% plasticized and coplasticized TPS films were assessed after equilibrate in 100%RH for 1 day (Figure 8). Due to the brittle nature, a starch S.D could not be tested.

Flexibility of the created TPS films increased in the order I.PS $<$A.PS$<$ M.PS $<$ MA.MD.PS $<$ MA.G.PS $<$ MA.U.PS. Increase in hydrogen bond capability per molecule of plasticizer increases the strain at break but decreased the crystallinity and tensile strength of TPS films. More hydrogen bonding functionalities increased the capabilities of the plasticizer to disrupt starch inter- and intramolecular hydrogen bonds and to absorb moisture. Isoleucine plasticized samples were relatively strong but brittle due to increased film crystallinity.

The results of coplasticized formulations were comparable; they showed high elongation at break, low tensile strength and low moduli. It indicated that malic acid in coplasticizer influenced esterification and urea, glycerol, and maltodextrin interfered with the remaining starch–starch hydrogen bonds. Hence, strain was limited by starch crosslinks. Unreacted 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 work[18]: 30G.PS, 30U.PS, and 30MA.PS. This was attributed to glycerol and urea being effective plasticizers for starch, disturbing starch inter- and intramolecular hydrogen bonds. The MA.MD.PS coplasticized sample showed lower elongation but same strength compared to urea and glycerol coplasticized samples. This could be due to crystallinity and poor plasticizer nature of maltodextrin itself. In previous work,[8] maltodextrin showed poor plasticizer characteristics due to less interaction between starch chain and maltodextrin, resulting in 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 coplasticized starch films is reported in another research,[13] similar as reported in the present study. It was thought that, in this study, the coplasticizer (glycerol, urea, and maltodextrin) effectively
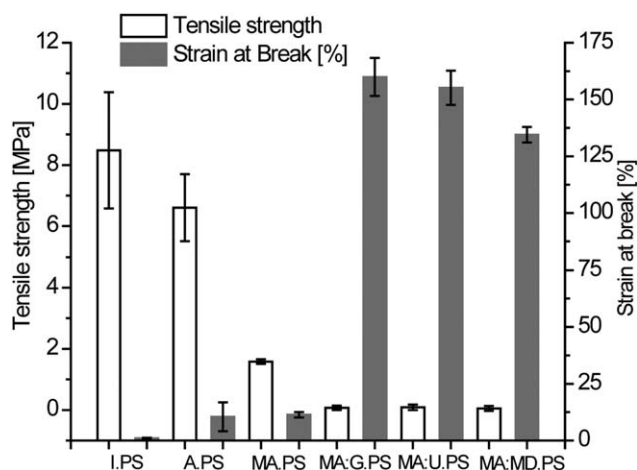
Figure 8. Tensile properties of the acquired TPS films.

disrupts hydrogen bond interactions between starches, whereas malic acid gives rise to partial esterification/crosslinking of starch limiting strain 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 coplasticized) 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 nontransparent.

Retrogradation was suppressed for the malic acid and coplasticized 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 crosslinking and strong hydrogen bonding between starch and malic acid is considered to prevent the observed behavior of the starch plasticizer mixtures.

Isoleucine showed poor interactions with starch and segregated during spray drying, limiting the plasticizing effect of isoleucine. 30I.PS 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 was recorded for any of plasticizer except for the asparagine plasticized formulation. All films are stable up to 160°C, crystallinity

and retrogradation behavior is correlated with strength and moisture absorption is related to the extent of strain.

Future work needed to be done on the selection of plasticizer that can increase the hydrophobicity of TPS blends alone or in combination with malic acid.

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