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RESEARCH ARTICLE

Oxidized potato starch based thermoplastic films: Effect of combination of hydrophilic and amphiphilic plasticizers

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Different combinations of hydrophilic (glycerol and water) and amphiphilic (isoleucine) plasticizers were studied in the production of thermoplastic starch (TPS) powders and films from oxidized potato starch. All powder samples had an irregular and shrivelled morphology. In all mixtures containing isoleucine, this additive exuded from the starch matrix and re-crystallized during spray drying, resulting in hollow particles. The crystallinity and moisture sensitivity of freshly prepared films depended on the isoleucine content in the plasticizer formulation. Increasing the content led to a decrease in moisture absorption. Isoleucine formulated films showed the best tensile strength, whereas glycerol-rich ones showed better strain at break values. All glycerol plasticized formulations and the spray dried non-modified starch were amorphous. Glycerol reduced the glass transition temperature to 128°C and glycerol formulated films showed the highest moisture uptake due to its hydrophilic nature. At a relative humidity (RH) of 50%, the rate of retrogradation was low, but at RH 100% all TPS films showed a high rate of retrogradation.

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1 Introduction

Petroleum-based plastic waste causes severe environmental problems and therefore gained considerable attention. The non-biodegradable nature of these plastics not only caused water contamination, but also adds to global warming [1]. Significant use of plastics and fluctuating price of petroleum-based resources triggered researchers to develop biodegradable and renewable plastics using biodegradable polymers such as poly(lactic acid) (PLA) [2], poly(hydroxyalkanoates) (PHAs) [3], and thermoplastic starch (TPS) [4]. TPS has become a popular choice for non-durable plastic-related industries, especially in food packaging [4, 5],

as it is readily available from different renewable resources [6]. Starch is a polysaccharide consisting of amylose (linear 1,4-linked α -D-glucan) and amylopectin (1,6- branched α -D-glucan). For the manufacturing of TPS films and sheets, starch is plasticized with water in combination with low molecular weight plasticizers, that is, urea, glycol, glycerol, threitol, xylitol, glucose, or maltose [7, 8]. These plasticizers interact with the starch backbones through hydrogen bonding. The processing of these mixtures is generally done through a combination of low to high shear “dry mixing” in tumblers or planetary mixers [9] followed by calendaring, sheet extrusion or pellet compression [10] and can be directly mixed and extruded [6]. TPS have shown good oxygen barrier properties [11], biodegradability [12], and compostability [5], but its strong water absorption [13–15] induces recrystallization which results in the loss of mechanical properties such as brittleness and tensile strength [16].

Retrogradation is promoted by hydrophilic plasticizers and water. To overcome this “disturbing” influence of, in particular, water and maintaining plasticizer activity during the manufacturing process, another thermal processing route was established, that is, “solution spray drying (SSD).”

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Abbreviations: DSC, differential scanning calorimetry; PHAs, poly(hydroxyalkanoates); PLA, poly(lactic acid); RH, relative humidity; SEM, scanning electron microscopy; TPS, thermoplastic starch

In SSD, factors such as short drying time, high diffusion rate and low moisture contents of the end product helped to suppress the retrogradation of TPS blends and produced amorphous TPS films [13, 14, 17]. Nevertheless, the rates of retrogradation and moisture adsorption (at different humidity levels) were found to be dependent on the type of plasticizer used. Low molecular weight hydrophilic plasticizers showed high adsorption rates, thus affected the rate of retrogradation [1, 13]. Therefore the rate of moisture uptake and the chemical structure of plasticizers are connected and too a large extent determines mechanical properties and retrogradation of TPS [7, 17–20]. SSD followed by compression molding was successfully used to produce TPS films formulated with natural plasticizers [13], amino acids [14], carboxylic acids [14, 21], and malto-oligosaccharides [17]. Isoleucine plasticized films showed low moisture uptake and high tensile strength, whereas films containing glycerol showed opposite behavior [13, 14].

Against this backdrop a study was undertaken with the aim to produce TPS films with improved mechanical properties and controlled moisture adsorption. Isoleucine is a natural amino acid and is amphiphilic. In previous work [14], it showed to combine good resistance to moisture uptake with significant film strength but also showed a high rate of retrogradation due to its own recrystallization.

As a follow-up on previous work with plasticized amorphous starch, the combination of amphiphilic and hydrophilic plasticizers in the starch matrix is expected to provide a tool to control and balance physical and mechanical properties. This paper presents a study on the co-plasticization and production of TPS films by mixing isoleucine and glycerol in different ratios and evaluating the effect of the composition on the rate of retrogradation, moisture adsorption and mechanical properties. Oxidized potato starch was used as a feedstock for SSD [19]. Different end-product properties of powders and films were investigated, with special focus on the rate of retrogradation, moisture adsorption and mechanical properties of TPS films. In addition, thermal properties and morphology of TPS powders were studied and analysed by differential

scanning calorimetry (DSC), thermal gravimetric analysis, and scanning electron microscopy, respectively.

2 Materials and methods

2.1 Materials

Oxidized potato starch (moisture content 15%) was supplied by AVEBE (the Netherlands). Analytical grade glycerol and L-isoleucine (reagent grade) were purchased from Sigma-Aldrich. Applied code names are given in Table 1. The percentage in the code name indicates the weight percentage of plasticizer used to dry starch weight.

2.2 Preparation of TPS-powder

An aqueous solution of starch was prepared using 15% (w/w) of starch and specified amounts of plasticizer (Table 1). These mixtures were heated at 95°C for 25 min applying a stirring rate of 600 RPM to prepare homogeneous solutions.

The obtained solutions were fed to a Büchi mini spray drier B-191 equipped with a 0.7 mm nozzle. A similar procedure was adopted as described in literature [6, 13] for the production of amorphous powder using plasticizers.

2.3 Preparation of TPS-films

TPS films (diameter of films are $\varnothing 10 \times 0.5 \text{ mm}^2$) were obtained by compression moulding the TPS powder, using a Fontijne Holland Table Press TH400. Samples were compressed at 25 bars for 5 min. The moulding temperature for all samples was 140°C.

2.4 Scanning electron microscopy (SEM)

A Jeol 6320F Scanning Electron Microscope was used to acquire insight in the morphology of the spray-dried particles. The applied accelerating voltage for

Table 1. Code names and constant values (k_1 and k_2) and coefficient of determination (r^2) for sorption curve Eq. (2) (Peleg model) of TPS films at 100% RH

Code	Description (w/w of starch)	M_0	k_1	k_2	r^2
U.S	Feedstock material	NA	NA	NA	NA
S.D	Spray dried starch	3.5	5.4×10^{-2}	1.7×10^{-2}	0.99
30G.PS	30% glycerol plasticized starch	1.2	2.2×10^{-2}	8.6×10^{-3}	0.99
30I.PS	30% isoleucine plasticized starch	2.5	7.3×10^{-2}	1.4×10^{-2}	0.99
(2:1)G.I.PS	20% glycerol and 10% isoleucine plasticized starch	0.8	2.1×10^{-2}	8.5×10^{-3}	0.99
(1:1)G.I.PS	15% glycerol and 15% isoleucine plasticized starch	1.1	2.4×10^{-2}	8.3×10^{-3}	0.99
(1:2)G.I.PS	10% glycerol and 20% isoleucine plasticized starch	0.8	3.7×10^{-2}	1×10^{-2}	0.99

NA, not applicable.

measurement was 3.0 kV. Samples were covered with a thin palladium/platinum conductive layer created with a Cressington 208 sputter coater before analysis.

2.5 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. The same procedure as mentioned in literature [13, 14] was adopted without any modification.

2.6 Thermal gravimetric analysis (TGA)

Open pan TGA of freshly produced TPS powder was performed in a Perkin Elmer TGA. 7.5–10 mg of each sample was tested from 25 to 900°C applying a heating rate of 25°C/min. The derivative of the weight loss curve was determined using the software program Origin 8.1, producing the DTGA-curve. The decomposition temperature (T_{dec}) was determined using the method applied by Soliman et al. [22].

2.7 Differential scanning calorimetry (DSC)

The Glass transition temperature (T_g) of freshly produced TPS powder 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 to 200°C. Open pan measurement was performed in order to remove any remaining water from all samples in the first run.

2.8 Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared analyses 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.

2.9 Retrogradation and moisture uptake

Retrogradation was studied by storing TPS films (diameter of Ø10 × 0.5 mm²) at three different relative humidity conditions: 0% (dried silica), 50% (35.64% CaCl₂ solution), and 100% (distilled water). 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 used as described in section X-ray diffraction.

The degree of crystallinity was determined using the method described by Wang et al. [23]. The same procedure as explained in previous work [13, 17] was used for integration and calculation of crystallinity. To study moisture uptake, films stored at 0, 50, and 100% RH were gravimetrically analyzed. Measurements were taken everyday during the first 7 days. Hereafter measurements were performed at day 14, 21, 28, and 56. Moisture uptake of the films was determined using Eq. (1) [24].

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

The subscript “day,*n*” denotes the weight at the day of measurement, “day,0” indicates the weight directly after film making. Moisture adsorption data were fitted according to the model reported in literature called Peleg model (Eq. 2) [25].

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

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) [26]. The Peleg parameters [25], k_1 and k_2 are shown in Table 1. 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 [26]. The coefficients of determination were found to be very high in all cases ($r^2 > 0.99$).

2.10 Mechanical testing

Mechanical properties of the TPS films were measured using an Instron 4301 tensile tester in accordance with ASTM D1708. The crosshead speed used was 10 mm/min. Before measurement, the test specimens were conditioned at RH = 100% for 24 h at 25°C. At least seven samples were tested for each specimen.

3 Results and discussion

3.1 Powder morphology

Surface morphology of TPS powder granules was examined by using SEM. Figure 1 shows the morphology of unplasticized starch and plasticized SSD powder samples. Morphology of powder granules was similar to previous results [14]. The feedstock U.S material displayed spherical and oval shaped particles of irregular sizes. Visibly different particle morphology was observed after SSD. Particles were spherical in shape however they contained uneven surface. Hollow particles were also produced. Both features are typical for spray dried powders [27, 28]. The drying sequence

U.S (Feedstock material)

Figure 1. Morphology (SEM) of plasticized spray dried starch powder.

determines the degree of shrinkage. The diffusion of moisture from particles leads to the formation of dents and hollows in the particle surface [6, 28]. Thirty G.PS had a morphology similar to that of S.D. Isoleucine plasticized and co-plasticized samples manifested a different morphology (Fig. 1). The differences were more pronounced for increasing isoleucine concentrations with broken hollow particles observed for high isoleucine concentration in plasticizer. The formation of hollow and broken particles was also observed previously [14]. Isoleucine is “hydrophobic” in nature and was incompatible with the moisture-rich starch droplet during the drying step [14]. As a result isoleucine was transported to the outside of the particle where it rapidly formed a crystalline barrier for the evaporating moisture.

The tendency to form broken and hollow particles is directly proportional to the isoleucine concentration in the formulations: 30I.PS > (1:2)G.I.PS > (1:1)G.I.PS, while no hollow particles are observed for the formulation with the highest glycerol content, (2:1)G.I.PS.

3.2 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 Fig. 2. Analysis showed that isoleucine plasticized and co-plasticized powder formulations showed crystallinity (Fig. 2a). These peaks were assigned to isoleucine crystalline domains. Pure

Figure 2. XRD patterns of freshly prepared, a) Powders b) TPS films.

isoleucine showed XRD peaks at $2\theta = 6.3^\circ$, 12.6° , 25.4° , 31.9° , and 38.6° (Fig. 2a). In isoleucine plasticized and co-plasticized samples these peaks were visible in the diffractograms and intensity of these peaks depended on the isoleucine concentration in plasticizer. 30I.PS and (1:2)G.I.PS gave higher intensity peaks than (1:1)G.I.PS and (2:1)G.I.PS. Similar results were observed in literature [14]. Additionally, XRD analysis supported the presence of isoleucine crystals in SEM analyses. On the other hand, 30G.PS and S.D powders were completely amorphous. Results are in line with previous work, that is, crystallinity peaks [6, 13, 14, 17]. The B- and V-type crystalline peaks at $2\theta = 17.2^\circ$, 19.6° , 22.3° , and 23.6° in U.S were completely absent after SSD (Fig. 2a) [29, 30]. Short drying times prevented the association of starch chains into crystal lattices [6].

For the films, XRD analysis of freshly prepared samples demonstrated that S.D and 30G.PS samples were

amorphous (Fig. 2b). By comparing Fig. 2a and b, it was clear that compression molding did not affect the amorphous character of the samples. Isoleucine plasticized and co-plasticized films again showed crystalline peaks, attributed to isoleucine (Fig. 2b).

The amorphous structure of the 30G.PS films and the crystalline one of isoleucine plasticized and co-plasticized films was further illustrated by the transparent, respectively, mat nature of the films [14].

3.3 Thermal properties

Thermal analyses showed different weight loss profile for all the samples (Fig. 3). 30I.PS and co-plasticized formulations showed similar decomposition patterns. Loss of water and other volatiles occurred up to 110°C (section a). Subsequently, decomposition due to the presence of plasticizer was

Figure 3. TGA and DTGA-curves of solution spray dried amorphous plasticized powder samples.

observed (sections b_1 and b_2). $T_{dec,b1}$ was associated with glycerol decomposition and $T_{dec,b2}$ to isoleucine. Both decomposition temperatures increased with lowering the amount of glycerol plasticizer contents in mixture. The main degradation zone for starch was observed in section b_3 . Section c indicates the propagation zone, leading to carbonaceous residues [22, 31]. U.S, S.D, and 30G.PS had shown strange decomposition pattern. U.S showed two decomposition temperatures, that is, first at 255°C and second at 318°C. Similarly, S.D and 30G.PS showed two and three decomposition temperatures, respectively. In 30G.PS, the first decomposition temperature (b_1) was associated with glycerol plasticizer and b_3 with starch degradation zone. The unique decomposition b_2 was observed in U.S, consequently present in S.D and 30G.PS.

The thermal properties of co-plasticized formulations depend on the ratio in which plasticizers used. (2:1)G.I.PS showed the lowest T_{dec} and (1:2)G.I.PS the highest, that is, 175 and 185 °C, respectively. This clearly proves the different nature of glycerol as plasticizer compared to isoleucine and the variation in interaction as a function of the chemical structure of the plasticizer. This also showed the poor plasticization characteristic of isoleucine as discussed in literature [14]. The largest fraction of the starch-plasticizer formulations decomposes at the temperature defined as $T_{dec,b3}$. It was found that the addition of plasticizer decreased this temperature, but did not show any effect of co-plasticizer ratio. All the plasticized fractions had $T_{dec,b3}$ within range of 306–308°C. $T_{dec,b1}$ and $T_{dec,b2}$ showed regular pattern with the quantity of glycerol in co-plasticizers, that is, decrease in decomposition temperature was observed with increasing glycerol contents. (2:1)G.I.PS had lowest $T_{dec,b1}$ and $T_{dec,b2}$ temperatures in co-plasticizers, that is, 191 and 232°C, respectively. Whereas, (1:2)G.I.PS had the highest $T_{dec,b1}$ and $T_{dec,b2}$ temperatures, that is, 217 and 241°C, respectively.

The results showed that glycerol and isoleucine embedded in starch matrix act differently as plasticizer. Glycerol seems to blend more homogeneously with the starch matrix and forms molecular blends with specific thermal

degradation characteristics. Comparing the behaviour of all the formulated blends, it may be concluded that isoleucine refrains from molecular mixing and forms a separated phase within the plasticized starch matrixes.

3.4 Glass transition temperature (T_g)

DSC results showed no obvious information regarding the T_g of the materials except for the 30G.PS formulation. Different combination of glycerol and isoleucine were used as plasticizer for TPS. Among all tested plasticizers, only addition of 30% glycerol resulted in reduction of T_g up to 128°C. All other used combination did not show T_g . The decrease of T_g in case of 30% glycerol can be attributed to disruption of hydrogen bonds between starch molecules due to plasticizer molecules. Isoleucine tends to phase separate from starch (vide supra) and did not show T_g . In co-plasticized formulations, the quantity of glycerol was not enough to show T_g of blends. S.D showed no T_g which was attributed to the strong inter- and intra-molecular hydrogen bonds between the starch chains limiting chain mobility [10].

3.5 FT-IR analysis

Characteristic absorption bands of the TPS samples were presented in Table 2. Absorption of starch occurred similarly as reported in literature [13, 17]. The broad band at 3334 cm^{-1} was assigned to O–H stretching [32–34]. C–H stretching was assigned to the peaks at 2919–2915 cm^{-1} [32]. Absorption at 1150–1149 cm^{-1} and 1078 cm^{-1} was attributed to C–O stretching in C–O–H [35, 36]. Finally, bands at 1019–1018 cm^{-1} were allocated to C–O stretching from C–O–C [35–37].

The effect of spray drying on S.D was in line with the previous studies [13, 14, 17]. Comparing the spectrum of U.S and S.D, it can be seen that the interactions with O–H, C–O–H, and C–O–C changed as a result of spray-drying. Low moisture contents and reduction in crystallinity could be the reason for these changes [29, 38].

Table 2. Characteristic absorption bands in starch and TPS samples

Samples	Wavelength [cm^{-1}]					
	3310–3290 (O–H stretching)	2922–2921 (C–H stretching)	1630–1607 (N–H deformation)	1149–1148 (C–O stretching)	1078 (C–O stretching)	1019–1017 (C–O bond stretching)
U.S	3290	2933	—	1149	1078	994
S.D	3298	2918	—	1149	1077	1017
30G.PS	3275	2924	—	1150	1079	1018
30I.PS	3248	2902	1609	1149	1078	1021
(2:1)G.I.PS	3246	2925	1610	1150	1079	1020
(1:1)G.I.PS	3275	2919	1609	1150	1079	1021
(1:2)G.I.PS	3266	2926	1609	1150	1079	1021

The absorption band at $1017\text{--}1021\text{ cm}^{-1}$ was clearly visible for all plasticized samples, indicating low crystallinity [38]. The short band in the range of $2908\text{--}2930\text{ cm}^{-1}$ was related to CH stretching in starch. This band was double peaked after using plasticizers in contrast to the single peak observed in native starch, implying the formation of newer and stronger hydrogen bonds [39]. All spectra showed shifts in the fingerprint region of starch, indicating interaction between starch and additives. But shift did not show any significant variation among different formulations. The O–H stretching vibration decreased, that is, 3298 cm^{-1} (S.D), for all the plasticized formulation. Hence, plasticizer gave rise to increased interactions with starch. The band at 1615 cm^{-1} was assigned to N–H deformation and was only appeared in isoleucine plasticized and co-plasticized formulations [32, 40, 41].

3.6 Retrogradation and moisture uptake

Film retrogradation, measured by X-ray diffraction, was studied over a period of 56 days at various humidity conditions, that is, RH0, 50 and 100% (Fig. 4a). 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 [18, 42], relative

weight changes during storage were also measured (Fig. 4b). All films showed an increase in the final crystallinity levels and relative weights in the order of RH 100% > RH 50% > RH 0% (Fig. 4a and b).

At RH 0% all films maintained similar crystallinity levels and remained brittle (Fig. 4a) due to the absence of water. The plasticizer content showed hardly any influence on recrystallization over time. Of all the formulations, 30G.PS and S.D were completely amorphous. Other formulation had shown crystallinity between 6 and 8%, which was attributed to the crystallinity of isoleucine itself.

At 50% relative humidity the films increased in weight over time, and finally reached an equilibrium plateau and consequently, this increase in moisture absorption had little effect on the extent of retrogradation. The percentage increase in moisture uptake and crystallinity were maximum in glycerol rich formulations attributed to hydrophilic nature of glycerol. Films became flexible due to moisture absorption and water acting as a plasticizer [13, 14]. Initially, the amorphous and dry starch matrix absorbs water, resulting in an increase in weight [43, 44]. This water acts as a plasticizer forming starch-water interactions, thus creating a more open molecular network. Finally, upon a further increase of the water contents structural rearrangements can occur, resulting in recrystallization [43]. Isoleucine plasticized samples

Figure 4. (a) Degree of crystallinity of films after 56 days of storage at different humidity levels, (b) relative weight change on different humidity levels of all plasticized TPS films.

Figure 5. Water sorption performance at 100% RH for 30% plasticized and co-plasticized formulations fitted using Peleg model (X7 and X56 are the degree of crystallinity at day 7 and day 56, respectively).

exhibited an increase in degree of crystallinity over time, attributed mainly to Isoleucine crystals. Diffraction peaks of isoleucine were in the area $2\theta = 12^\circ\text{--}31^\circ$ (Fig. 2). Consequently, the overall degree of crystallinity in isoleucine plasticized and co-plasticized samples were influenced by isoleucine crystallization and glycerol hydrophilic nature, respectively. 30I.PS, (1:2)G.I.PS and (1:1)G.I.PS samples remained brittle upon storage.

At 100% relative humidity the differences between the formulations became more evident. The rate of moisture absorption and the ultimate equilibrium moisture levels were clearly the highest for the glycerol formulated starches. 30G.PS, (2:1)G.PS, and (1:1)G.PS had shown maximum moisture uptake, however 30I.PS and S.D tend to repel moisture ascribed to hydrophobic nature of both. But the rate of retrogradation did not follow this pattern and all formulations showed significant recrystallization, attributed to hydrophilic nature of glycerol and formation of isoleucine crystals.

To better understand water sorption performance at 100% RH, the moisture data at different times were fitted using Peleg model, Eq. (2) (Table 1) [25].

Figure 5 presents an overview of the data for the 30%w plasticized single formulations including the crystallinity data at day 7 and day 56. The coefficients of determination were found to be very high in all cases ($r^2 > 0.99$). 30G.PS showed the highest initial adsorption rate. S.D and 30I.PS had similar initial and final adsorption rate. The absence of hygroscopic plasticizer in S.D and hydrophobic nature of isoleucine resulted in the lowest value for the initial adsorption rate.

Figure 5 illustrates the results of glycerol and isoleucine co-plasticized formulations at 100% RH. Glycerol-rich

formulations had high moisture absorption attributed to hydrophilic nature of glycerol. All three co-plasticized samples approach the equilibrium state after 28 days. (2:1) G.I.PS and (1:1)G.I.PS showed similar moisture absorption for 56 days, whereas (1:2)G.I.PS showed low absorption attributed to high isoleucine contents in co-plasticizer.

3.7 Mechanical properties

Mechanical strength is one of the most important parameter to evaluate especially for used in packaging application. The mechanical behaviour of 30% plasticized and co-plasticized TPS films were assessed after equilibrate in 100% RH for 1 day Fig. 6 and Table 3. Due to the brittle nature of starch S.D could not be tested.

Figure 6. Mechanical properties of the TPS films.

Table 3. Degree of crystallinity, relative weight, thermal properties, and mechanical properties of TPS films

Formulations	Thermal properties (°C)				Mechanical properties				Degree of crystallinity of films			Relative weight change films					
	Moisture contents powder (%)	T_{dec}	T_{dec}		$T_g^{a)}$	T_{dec}	$b_2^{a)}$	b_1	Modulus ^{a)} (MPa)	Tensile strength ^{a)} (MPa)	Strain ^{a)} (%)	0 day (%)	7 days	56 days	RH 100%	1 day	56 days
			T_{dec}	b_3													
U.S	15 ± 1	241	255	n.o	314	n.o	n.o	b	b	b	b	b	b	b	b	b	b
S.D	3.48 ± 1	235	253	n.o	318	n.o	n.o	a	a	a	a	0	8.9 ± 0.3	16.4 ± 0.5	b	b	b
30G.PS	1.21 ± 0.5	150	186	240	308	128 ± 0.2	307	44.1 ± 3	1.1 ± 0.2	1.1 ± 0.2	19 ± 1	0	14.5 ± 0.3	17.3 ± 1	b	21.9 ± 1	61.6 ± 2.5
30I.PS	2.56 ± 0.3	199	234	n.o	307	n.o	305	1031 ± 20	10.5 ± 0.8	10.5 ± 0.8	1.7 ± 0.2	8.2 ± 0.3	14.3	19.4 ± 0.5	b	36.3 ± 2	111.3 ± 4
(2:1)G.I.PS	0.8 ± 0.2	175	191	232	305	n.o	306	455 ± 10	6.4 ± 0.5	6.4 ± 0.5	3.9 ± 0.4	6.3 ± 0.2	11.1	16.2 ± 0.6	b	27.2 ± 1.5	70.7 ± 2.5
(1:1)G.I.PS	1.13 ± 0.5	177	191	239	306	n.o	306	326 ± 8	6.9 ± 0.4	6.9 ± 0.4	7.2 ± 0.3	6.7 ± 0.2	11.9	16.4 ± 0.7	b	37.2 ± 3	112.8 ± 2.5
(1:2)G.I.PS	0.81 ± 0.3	185	217	241	306	n.o	306	642 ± 9.5	10.3 ± 0.4	10.3 ± 0.4	2.8 ± 0.2	5.7 ± 0.3	11.9	18.9 ± 0.5	b	34.1 ± 2	116.5 ± 2.5
																22.6 ± 1	91.4 ± 3

a) a: S.D could not be tested due to brittleness of the test sample; b: did not test; n.o: not observed.

Glycerol plasticized films showed higher elongation, whereas isoleucine containing films had superior strength. The results were in line with previous work [13, 14], where 30G.PS formulated films showed better plasticization and elongation than the ones plasticized with 30G.PS. Increasing hydrogen bond capabilities per molecule reduced crystallinity and tensile strength, but increased strain. The capability of plasticizer to disrupt starch inter- and intra-molecular hydrogen bonds mainly depend on hydrogen bonding functionalities of it. Hereby films plasticized with glycerol showed better chain mobility and thus flexibility. Hereby glycerol improved starch chain mobility and thus flexibility. Isoleucine plasticized samples were relatively strong but brittle due to increased film crystallinity [14].

Similar results were obtained for co-plasticized films. The Flexibility of the created co-plasticized TPS films increased in the order (2:1)G.I.PS > (1:1)G.I.PS > (1:2)G.I.PS. The co-plasticized sample showed lower elongation and strength compared to 30G.PS and 30I.PS samples. This could be due to crystallinity and poor plasticizer nature of isoleucine itself.

The co-plasticized sample showed lower elongation and strength compared to 30G.PS and 30I.PS samples. This could be due to crystallinity and poor plasticizer nature of isoleucine itself. The study proved the poor plasticizer characteristics of isoleucine with poor interaction between starch chain and isoleucine, had no T_g and high rate of retrogradation.

4 Conclusions

Combinations of hydrophobic (glycerol and water) and amphiphilic (isoleucine) plasticizers in the production of TPS films were studied.

Freshly prepared powders and films containing isoleucine were crystalline. Isoleucine-rich plasticized TPS powders and films exhibited crystal structures attributed to crystallisation of isoleucine and were non-transparent. TPS powder granules were irregular and shrivelled with high rate of agglomeration. The content of hollow particles observed in the powders could be related to the isoleucine concentration in the formulation.

Significant retrogradation for all TPS formulations was observed for 56 days at 50% RH and 100% RH. Rate of retrogradation was high during the initial 7 days and reached a maximum after 28 days. No T_g was recorded for any formulated samples except for 30G.PS. Isoleucine showed brittle behavior and the highest strength, whereas glycerol rich samples showed the highest strain at break.

Isoleucine could be dissolved and mixed into aqueous starch solutions but segregated during spray drying and caused the formation of low bulk density hollow powder particles. Due to this crystallization and the poor interaction

with the starch matrix, isoleucine is clearly not a proper plasticizer for TPS films.

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