Highly flexible polylactide food packaging plasticized with non-toxic, bio-sourced glycerol plasticizers

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Abstract

Polylactide (PLA) is a promising bio-sourced and biodegradable polymer substitute for traditional petroleum-based products. Despite its recognized potential, its widespread adoption is restricted by its brittleness and low ductility and, thus, to enhance its material properties, plasticizers must be blended with PLA to lower the glass transition temperature (T_g) and impart flexibility into the blend. As such, this work focused on the synthesis of a family of bio-sourced plasticizers for applications in flexible food packaging using glycerol, succinic anhydride, and alcohols of varying chain lengths. The effect of chemical structure on plasticization performance, migration, blend morphology, and toxicity were evaluated and compared to the commercial plasticizer acetyl tributyl citrate (ATBC). Plasticizer/PLA blends were prepared using solvent-casting as well as melt-mixing to produce thin films and bulk specimens. At loadings of 20 wt%, improved flexibility (up to 435% elongation) was observed in films with the glycerol plasticizers relative to neat PLA (6% elongation), while T_g's were reduced by up to 45 °C from that of neat PLA (T_g ~ 60 °C). Phase morphologies evaluated with SEM showed good incorporation of the plasticizers into the PLA matrix. Leaching behaviour of the plasticized blends were evaluated in different food simulants and showed that plasticizers comprised of branched, or longer alkyl chains produced 2- to 6-fold lower migration rates compared to those with short alkyl chains. Finally, plasticizer candidates were shown to be non-toxic and did impact HepG2 cell viability over a period of 7 days in an *in vitro* mammalian cell assay.

Key words: polylactide, biobased plasticizer, food packaging, toxicity, flexible packaging, glycerol

Introduction

A growing global awareness of the ramifications brought on by the manufacture, use, and accumulation of petroleum-derived plastics in the environment has resulted in significant interest in the development of sustainably sourced polymeric materials.[1] This has resulted in the development of a number of promising alternative bio-based polymers which have been shown to be both biodegradable and biocompatible.[2, 3] Although bio-based polymers currently account for only about one percent of the estimated 368 million tonnes of plastics produced annually, their market is projected to expand as the global production capacities are poised to increase from 2.11 million tonnes in 2020 to 2.87 million tonnes by 2025.[4] Within this group of alternatives, polylactide (PLA) has emerged as one of the leading replacements for conventional petroleum-derived polyethylene terephthalate (PET) and polystyrene (PS).[5] As a bio-sourced synthetic polymer, PLA is derived from animal and plant sources,[6] and has shown to be biodegradable under industrial composting conditions.[7] Having similar mechanical properties to both PET and PS,[8, 9] PLA has high tensile strength and modulus which make it suitable for certain packaging applications, 3D printing, and in the medical field for sutures and drug delivery owing to its biocompatability.[10-12]

Despite its wide scope of application, PLA suffers from several drawbacks including its brittleness, low ductility, and poor tensile properties.[13] These important drawbacks have limited its use in certain applications such as food packaging, for which high flexibility, elongation and toughness are essential.[12] One of the commonly-used approaches to modify the mechanical properties of PLA is through reactive blending between PLA and an immiscible rubbery polymer, such as polyethylene[14] or poly(1,4-*cis*-isoprene).[15] It has been demonstrated that this approach has the ability to significantly improve both the impact toughness as well as the ductility of PLA (ref). While this approach has considerable promise, it often requires the use of complicated synthetic procedures to selectively install reactive functional groups on the rubbery phase, which has, to some extent, limited its widespread use.[16] Alternatively, external plasticizers (herein simply referred to as plasticizers) which are blended with PLA can be used to help lower the glass transition temperature (T_g) of the polymer, impart flexibility, and improve processing characteristics is an industrially-adopted practice.[17, 18]

Although the addition of plasticizers to PLA is an accepted method to improve its mechanical properties, plasticizers are prone to migration and leaching out of the blend as they are not covalently bound to the polymer backbone.[18] This compromises the integrity of the product[19] and can lead to widespread environmental contamination and potential human exposure.[20, 21] Consequently, the investigation of plasticizer accumulation in the environment[22] as well as the health effects of plasticizer exposure on both humans[23, 24] and animals[25] remains an area of active research. To date, there have been numerous plasticizers developed for the production of flexible PLA including but not limited to citrate esters,[26] polyethylene glycol analogs,[27] levulinic acids,[28] tartaric acids,[29] malic acids,[30] and functionalized epoxidized soybean oils.[31] While many of these have demonstrated promise, the global push towards sustainable commodity plastics has resulted in the demand for alternative plasticizers synthesized from simple, renewably sourced feedstock chemicals.[32] Additionally, to mitigate risk while avoiding the regrettable substitution of one problematic plasticizer with another,[33] the toxicology of these alternative plasticizers must be taken into consideration.

In line with this, the goal of this work was to design a family of non-toxic bio-based plasticizers for flexible food packaging materials, while evaluating the effect of chemical structure on plasticization performance, surface morphology, and migration behaviour in blends with PLA. The green platform chemical, glycerol, was exploited as a building block to synthesize a series of glycerol-succinate bio-plasticizers functionalized with different alcoholic substituents which were then compared with the commercial standard plasticizer acetyl tributyl citrate (ATBC). In addition, we aimed to compare the thermal stabilities and surface morphologies of blends prepared using two different types of commonly employed preparation techniques (solvent casting of films vs. melt-mixing) to evaluate our family of plasticizers under both laboratory and industrial relevant conditions. Finally, the cytotoxicity of the plasticizers was investigated through an *in vitro* mammalian cell assay using Human Hepatocellular Carcinoma (HepG2) cells.

Experimental Methods

Materials and Reagents

Polylactic acid (Ingeo Bioworks 2003D, MFI = 6 g/10min (210 °C/2.16 kg) and density = 1.24 g/cm^3) was purchased from Nature-Works LLC (Minnetonka, MN). Succinic anhydride (99%), glycerol (99%), 2-ethylhexanol (99.6%), 1-butanol (99.8%), *n*-hexanol (99%), magnesium sulfate (99.5%), sodium bicarbonate (ACS reagent), and *p*-toluene sulfonic acid monohydrate (98.5%) were purchased from Sigma Aldrich (Oakville, ON). *n*-heptanol (99.9%) was purchased from Arkema (King of Prussia, PA). Ethyl acetate (ACS grade), *iso*-propanol (ACS grade), toluene (ACS grade), dichloromethane (ACS grade), acetic acid (99.5%), ethanol (ACS grade), water (LCMS grade), dimethyl sulfoxide (99%), Dulbecco's Modified Eagle Medium (DMEM), Penicillin Streptomycin solution, and Fetal Bovine Serum (FBS) were purchased from Fisher Scientific (Montreal, QC). Tributyl 2-acetylcitrate (ATBC) (98%) was used as a reference plasticizer and was purchased from Sigma-Aldrich (Oakville, ON). Cell-Counting Kit-8 (CCK-8) was purchased from Cedarlane Laboratories Ltd (Burlington, ON).

Synthesis of Plasticizers

See the appended Supporting Information for full experimental and characterization information regarding the synthesis of the plasticizer. Briefly, the appropriate mono-succinate (3.8 eq.) was reacted with glycerol (1 eq.) and *p*-toluene sulfonic acid monohydrate (0.03 eq.) under bubbling N_2 at 110 °C for 18 hours to afford the crude glycerol analogs as oils. Crude reaction mixtures were dissolved in ethyl acetate (50 mL), washed with saturated sodium bicarbonate (25 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford the glycerol-succinate (GS) analogs as clear to yellow oils in yields ranging from 82-95%. Each compound was named according to the alcohol substituent on the GS core.

Solvent-Casting of Films

Neat PLA (nPLA) and plasticized films were prepared using solvent-casting. PLA films were produced using 10 and 20 weight percent (wt%) of plasticizer using blends of 2.5 g total weight. Each blend was produced by combining the appropriate weights of PLA and plasticizer into a 50-mL round-bottomed flask and stirring at room temperature for 1 hour in 25 mL of dichloromethane. The films were then cast into a circular glass dish (diameter = 120 mm), covered with aluminum

foil, and left in a fumehood to dry for 48 hours. The films were then peeled from the dish and dried in a vacuum oven at 40 °C for 48 hours to remove residual solvent. The thickness of the obtained films was 0.14 ± 0.02 mm. Each blend was named according to the plasticizer weight percentage it is comprised of (i.e., a 20 wt% blend of ATBC is denoted as ATBC-20).

Melt Mixing of PLA/plasticizer blends

Melt-mixed blends at plasticizer concentrations of 10 and 20 wt% were prepared using a Rheocord System 40 double arm internal batch mixer (Haake Buchler). The PLA pellets were dried under vacuum for 24 hours at 40 °C to remove residual moisture before use. 50 g batches of each blend were pre-weighed and stirred by hand briefly before being added to the batch mixer and mixed for 10 minutes at 170 °C with a rotation rate of 50 rpm. The batches were then quenched into a liquid nitrogen bath to freeze the morphology and stored for further analysis. nPLA was processed under the same conditions for comparison purposes. Blends were named in the same manner as described above.

Scanning Electron Microscopy (SEM)

The morphology of the samples was investigated with scanning electron microscopy (SEM), using a FEI Quanta 450 Environmental Scanning Electron Microscope (FE-ESM) operating under high vacuum and acceleration voltage of 5.0 kV. The specimens were freeze-fractured with liquid nitrogen and mounted on aluminum stubs with carbon tape and glue. All samples were sputter-coated with 4 nm of platinum prior to analysis using a Leica Microsystems EM ACE600 High Resolution Sputter Coater.

Production of Tensile Bars

Tensile test bars of melt-mixed blends were produced using compression molding with a heated manual hydraulic press (Carver Manual Hydraulic Press with Watlow temperature controllers, St. Louis, MO) and steel molds. The blends were manually cut into small fragments and dried in a vacuum oven at 40 °C for 24 hours before being placed into the molds and pressed at 170 °C in the following manner: 5 minutes at 5 metric tonnes followed by 5 minutes at 10 metric tonnes. Tensile test bar dimensions adhered to the standardized testing protocol ASTM-D638 for tensile properties: i.e., a thickness (T_0) of 1.4 mm; a width of narrow section (W_0) of 3.3 mm; a length of narrow section of 17.8 mm; an overall length of 64 mm; and an overall width of 10 mm. The exact

dimensions (thickness/width) of each specimen were recorded using an electronic caliper prior to testing.

Thermal Gravimetric Analysis (TGA)

The thermal stability of the PLA blends was evaluated using a TA Instruments Discovery 5500 (New Castle, DE) instrument under nitrogen flow of 25 mL/min from 25 to 500 °C at a heating rate of 10 °C/min. The onset temperature at weight loss with 5% (T_5) is reported in the Supporting Information (see Table S1 and S2) for comparison purposes.

Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g) of each blend was measured by differential scanning calorimetry (DSC) using a TA Instruments Q2000 (New Castle, DE) under a nitrogen atmosphere using the following heat/cool/heat cycle. The samples were heated from 25-200 °C at a rate of 20 °C per minute, held constant at 200 °C for two minutes, then cooled to -30 °C at a rate of 10 °C per minute and held constant for two minutes at -30 °C. The samples were then heated to 200 °C at a rate of 20 °C per minute. The T_g was then determined from the reversible heat flow of the second heating cycle using the automated glass/step transition tool in the TA Instruments Universal Analysis 2000 software. The melting (T_m) and cold crystallization (T_{cc}) temperatures were taken from the second heating scan. The crystallinity (X_c) was calculated using equation (1) with the melt and crystallization enthalpies where ΔH_m, ΔH_{cc}, ΔH_m^0, and w_{PLA} represent the enthalpy of melting, the enthalpy of cold crystallization, the enthalpy of melting for 100% crystalline PLA, and the weight percent of PLA in the blend, respectively. A value of 93.15 J/g was taken as the melting enthalpy for 100% crystalline PLA.[34]

$$X_c = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0 \times w_{PLA}} \times 100\% \quad (1)$$

Tensile Testing

Testing was performed using a Shimadzu (Kyoto, Japan) Easy Test instrument equipped with a 500 N load cell in accordance with a previously developed protocol.[21] For the film specimens, samples were cut into rectangular strips with dimensions of 5 mm width x 60 mm length x 0.14 ± 0.02 mm thickness and stored in a desiccator until the testing was performed. Test strips were clamped and subjected to a strain rate of 20 mm/min with a constant gauge length of 30 mm. At

least five specimens were tested for each sample. *For the blended/compression molded samples,* test bars were clamped and subjected to a strain rate of 5 mm/min with a constant gauge length of 36 mm. The stress-strain curves were used to obtain values for strain (% elongation), stress at break, and the early extensional modulus (0-5% strain).

Plasticizer Leaching

The American Society for Testing and Materials (ASTM) Method D1239-14, "Resistance of Plastic Films to Extraction by Chemicals" was used as a template. Films blended with 20 wt% of plasticizer were used. Specimens were cut into square fragments measuring 2 cm x 2 cm and dried in a vacuum oven for 24 hours at 40 °C. Three food simulants were selected for leaching analysis: water, 10% v/v ethanol in water, and 3% v/v acetic acid in water over time points of one, five, and ten days at a temperature of 60 °C. Each specimen was pre-weighed, placed into a 24-mL glass vial containing 20 mL of each simulant and then placed into an incubator shaker set at 60 rpm for the specified time frame. Each experiment was run in triplicate. At the end of the time periods, the film specimens were removed from the vials, wiped with tissue paper, and dried in a vacuum oven at room temperature for 7 days. The film specimens were then re-weighed, and the percent mass loss was calculated using equation (2), as follows:

$$Mass\ Loss\ (\%) = \frac{W_i - W_f}{W_i} \times 100\% \quad (2)$$

where W_i is the initial mass of the specimen and W_f is the final mass

Cell Viability Assay

The human hepatocellular carcinoma (HepG2) cell line was used in this assay. Cells were thawed from stock and cultured using an established protocol.[35] After culture, the cell count was obtained with a Bio-Rad TC20 cell counter. Stock solutions were then prepared at different concentrations for the standard curve and test wells. The cell-counting kit (CCK-8), which uses a [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] salt (WST-8), was purchased and used according to the manufacturer's instructions. In brief, the WST-8 reagent produces a formazan dye upon reduction by a metabolically active cell to allow for a direct quantification of viable cells and evaluate cytotoxicity. HepG2 cells were collected and counted using a Bio-Rad TC20 cell counter. Cells were then plated in 96-well plates at a concentration of

5000 cells/well and incubated for 24 hours to allow for cell adhesion. The media was removed and 150 μL of sterilized solutions comprised of cell media and 500 μM of each plasticizer in 0.5% v/v DMSO (to improve solubility) were added to the appropriate wells and the plates returned to the incubator. A negative control of 0.5% v/v DMSO was added to the cells in the absence of plasticizer to account for toxicity of the solvent, while a positive control of 10% v/v DMSO was employed. Each solution was run in triplicate on the same plate. At time points of one and seven days, the media was removed, and 100 μL of media containing 10% v/v WST-8 reagent was added to each well and the plates returned to the incubator for two hours. After two hours, absorbance values at 450 nm were read using a Bio-Rad Benchmark Plus plate reader (CA, USA). Each absorbance value was normalized with respect to values obtained from wells containing cells seeded at 5000 cells/well. Each plate was analyzed in triplicate.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were collected using a Bruker AVIIIHD 500 MHz spectrometer (MA, USA) with an average of 16 scans using deuterated chloroform ($CDCl_3$) as the solvent.

Statistics

Statistical analysis was done using GraphPad Prism 5 software. Difference between the mean values of the plasticizer types were analyzed by a one-way-ANOVA test with a Bonferroni post-test to evaluate differences between each type. A p value less than 0.05 was interpreted as significant.

Results and Discussion

Synthesis of Plasticizers

A family of six glycerol-succinate analogs with different alcohol chain lengths were synthesized and evaluated as plasticizers for flexible PLA-based food packaging applications. Glycerol is an ideal building block for the design of green plasticizers as it is a non-toxic, renewably sourced chemical containing three alcohol functional groups for further synthetic manipulation. Here, glycerol was used as a platform molecule to design a series of succinate derivatives bearing different alcohol substituents to investigate the effect of alcohol chain length and substitution

pattern on plasticization efficiency, surface morphology, migration behaviour, and toxicity in blends with PLA.

Each analog was synthesized following the same two-step sequence (Figure 1). The appropriate mono-substituted acid was reacted with glycerol in the presence of catalytic amounts of *para*-toluene sulfonic acid (*p*TsOH•H_2O) under solvent-free conditions to afford the desired analogs as light yellow or clear oils. Initial attempts to esterify the secondary alcohol of glycerol were unsuccessful at reaction times of 4 hours. However, increasing the reaction time to 18 hours led to complete conversion and provided the fully esterified products. This approach avoids the use of organic solvent during the reaction, reaches high yields and conversions with very low catalyst loadings, and generates only water as a by-product during the esterification.

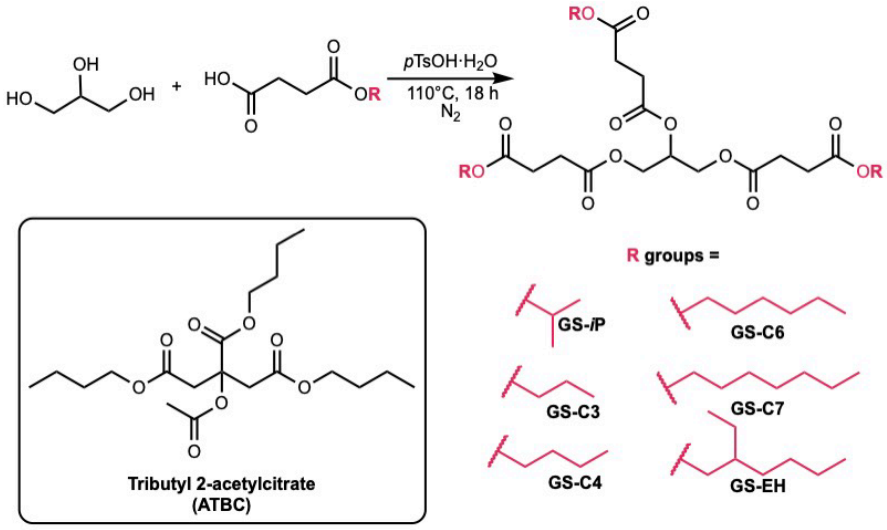

Figure 1. Synthesis and structures of glycerol-based plasticizers and commercially available ATBC used for comparison purposes in this study.

Theoretical Prediction of Compatibility of Glycerol Analogs with PLA

For a molecule to function effectively as a plasticizer, it must be both miscible and compatible with the host polymer system it is blended with.[36] In the case of a plasticizer-polymer system, this type of relationship can be evaluated with Hansen Solubility Parameters (HSP) in which theoretical predictions are made based on the chemical structure and molecular weight of the plasticizer in relation to the host polymer.[37] For a plasticizer to be deemed compatible, it must have similar solubility parameters to its host polymer as well as have a relative energy difference (RED) value of less than 1.[38] Here, the HSP values of each plasticizer candidate were calculated using the

Hoftyzer-Van Krevelen method[39] and compared to both PLA as well as ATBC (Table S5). Our calculated value of the solubility parameter for PLA of 20.6 $(MJ/m^3)^{1/2}$ was consistent with reported literature values which range from 20.1-21.9 $(MJ/m^3)^{1/2}$.[28, 40] The calculated RED values of ATBC and our plasticizer candidates ranged from 0.6-0.7, indicating that they were theoretically miscible with PLA.

Investigation as Plasticizers

In addition to investigating the plasticization efficiency of this class of bio-based plasticizers for PLA, we also evaluated two different types of commonly employed blend preparation techniques used in the literature (i.e., solvent casting of films versus melt-mixing). While solvent-casting is a generally-accepted method of sample preparation for small-scale screening of new plasticizers, this processing technique is not one which is used industrially.[41, 42] Therefore, we wanted to establish the efficiency of this class of plasticizers under both laboratory and industrially-relevant testing conditions while also comparing the thermal properties and surface morphologies of the blends arising from the two preparation techniques.

Thermal Properties of the Blends

To evaluate the plasticization efficiency of the compounds, films containing 10 and 20 wt% of each candidate plasticizer were prepared through solvent casting. In each case, flexible and transparent films were obtained after evaporation of the solvent. The thermal stabilities of the film blends were then evaluated with thermal gravimetric analysis (TGA) to evaluate the effect of alkyl chain length and branching on blend stability with the onset temperature at weight loss with 5% (T_5) reported for comparison (see Supplemental Information, Table S1). The film blends produced with the glycerol analogs displayed higher T_5 values at both 10 and 20 wt% (ranged between 244 to 293 for 10wt% and 195 to 271 °C for 20wt%) loadings (except for GS-C4-20) than blends produced with ATBC (T_5 of 222 and 207 °C for 10 and 20wt%, respectively). In comparison, the thermal stabilities of the melt-mixed blends prepared with the glycerol analogs also displayed higher thermal stabilities than blends produced with ATBC (see Supplemental Information, Table S2). In general, the thermal stability was higher for the analogs comprised of longer alkyl chains.

Each blend was then evaluated using DSC to obtain the glass transition (T_g), melting (T_m), and cold crystallization (T_{cc}) temperatures of the blends. The obtained DSC thermograms for film blends are presented in Figure 1. When compared to nPLA with a T_g of 59 °C, the glycerol analogs reduced the T_g between 22-26 °C at loadings of 10 wt%. A more pronounced decrease in T_g was found at 20 wt% plasticizer loadings where values as low as 15 °C were obtained for blends produced with the glycerol analogs. Between these, it was found that analogs comprised of longer, linear alkyl chains produced blends with the lowest T_g value observed at 20 wt% loadings, whereas the branched GS-EH did not produce the same desirable effect. As expected, the addition of the glycerol analogs slightly depressed the melting temperatures of the blends (see Supplemental Information, Table S3) relative to nPLA, while the increase in plasticizer loading from 10 to 20 wt% did not have a significant effect as comparable T_m values were obtained at both loadings. Cold crystallization was present in all the film blends, except for nPLA, ATBC-20 and GS-EH-10 and GS-EH-20 (see Supplemental Information, Table S4, for values), with the highest exotherms observed for the blends produced with the glycerol analogs comprised of C6 and C7 alkyl chains. In contrast, the DSC thermograms of the melt-mixed blends display slightly different thermal transition temperatures than those observed for the solvent-cast films (Figure S1); however, each glycerol analog effectively reduced the T_g of nPLA to between 25-36 °C, with GS-C4-20 displaying the lowest T_g of 24 °C.

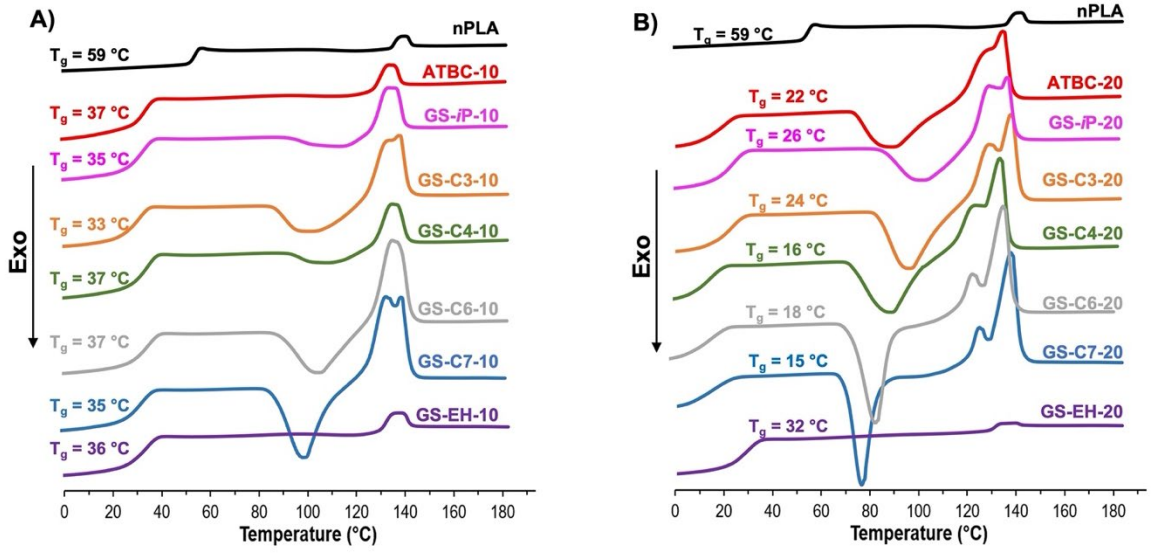

Figure 2. DSC thermograms of PLA film blends obtained from the second heating cycle with (A) 10 wt% and (B) 20 wt% glycerol plasticizers.

Mechanical Properties

Since PLA is known for its brittleness, low ductility, and poor tensile properties,[43] the addition of an effective plasticizer should enhance flexibility while increasing the elongation at break.[18] Tensile testing data of the solvent-cast film blends is summarized in Figure 3, with all plasticized blends showing an increase in elongation, and a decrease of modulus and stress at break compared to nPLA. Significant differences were observed in the modulus, stress at break, and elongation at break amongst the compounds tested at both 10 and 20 wt% plasticizer loading ($p < 0.001$). A representative stress versus strain in % elongation curve for 20 wt% plasticized films shows typical plasticizing behaviour for all blends, with the highest elongation of 435% obtained for GS-*i*P-20 (Figure 3A). An increase of plasticizer loading from 10 to 20 wt% did not have a significant effect on the elongation or stress at break for analogs comprised of alkyl chains greater than three carbons in length ($p > 0.05$). The early extensional modulus (0-5% strain) ranged from 71 MPa to 659 MPa for the 20 wt% blends, and from 221 MPa to 725 MPa for the 10 wt% blends. It has previously been shown that an elongation at break of 522% and a stress at break of 21 MPa were obtained with PLA films plasticized with 20 wt% epoxidized soybean oil methyl ester,[31] while an elongation at break of around 460% and a stress at break of 40 MPa were obtained using 20 wt% of a glycerol-based levulinic acid derivative.[28] Therefore, the obtained results for our glycerol-based succinates are comparable to previous literature reports as well as the commercial standard ATBC.

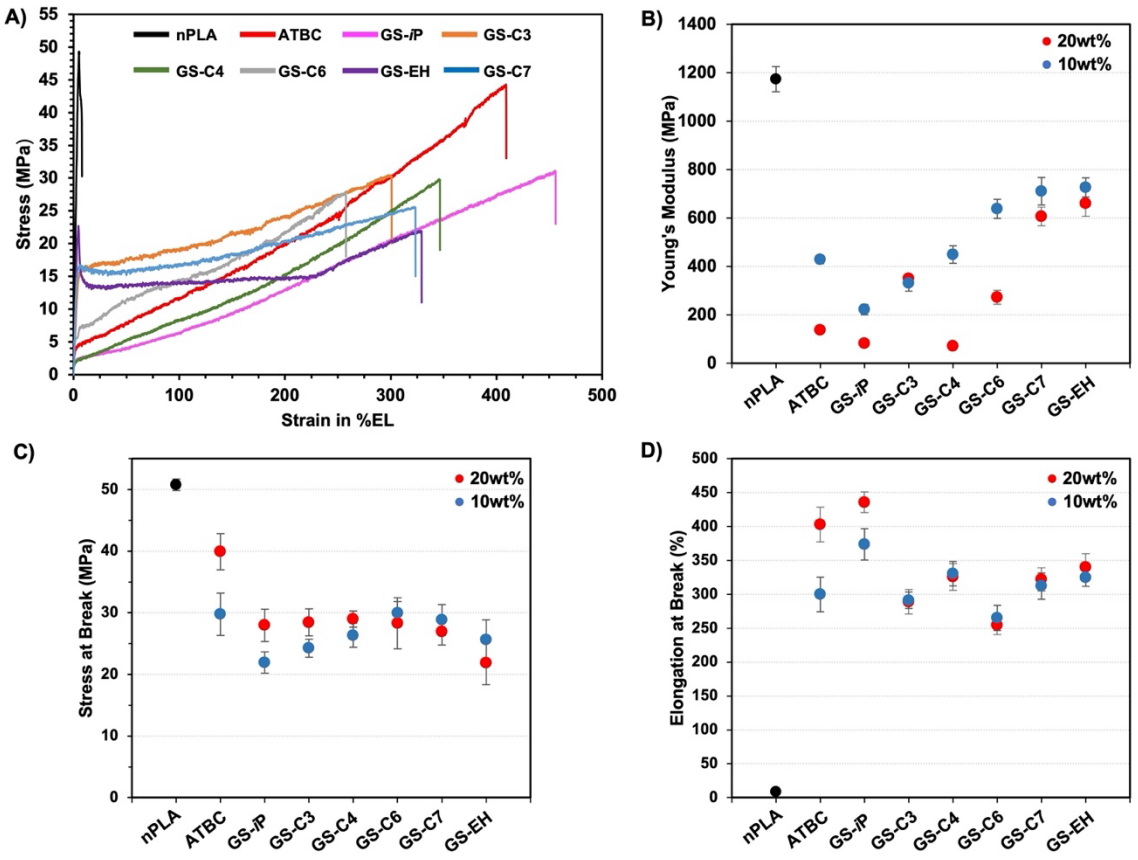

Figure 3. Mechanical properties of PLA film blends at 10 and 20 wt% plasticizer loadings: (A) Representative stress vs. strain in % elongation curves; (B) Young's modulus; (C) Stress at break; (D) Elongation at break (n=5, error bars represent standard deviation, means are shown).

The tensile testing data for the 10 and 20 wt% melt-mixed blends is summarized in Figure 4. Significant differences were observed in the modulus, stress at break, and elongation at break amongst the compounds tested at 20 wt% plasticizer loading ($p < 0.001$). In contrast to the solvent-cast films, the melt-mixed blends containing 10 wt% plasticizer displayed very poor tensile properties, with no significant differences observed in the modulus and elongation at break between any of the 10 wt% blends and nPLA ($p > 0.05$). Although the plasticizers and PLA form miscible blends at 10% loading, no improvement in mechanical properties was observed. This finding agrees with a previous report by Jeong and coworkers wherein the inability of their plasticizers to improve the tensile properties of PLA in bulk specimens at 10 wt% plasticizer loadings was also observed.[44] This is believed to be a direct result of antiplasticization,[36] in which low loadings of plasticizers produce blends with increased tensile strength, but decreased elongation. However, blends prepared with 20 wt% plasticizer loadings displayed remarkable

tensile properties. An increase in elongation of up to 257% for GS-C6 was obtained, while significant reduction in the modulus were observed for all 20 wt% blends (except for GS-EH). A representative stress versus strain in % elongation curve is presented in Figure 4A which shows standard plasticizing behaviour as the test bars displayed typical necking before fracture (see Supplementary Information, Figure S5). Interestingly, GS-EH was unable to improve the tensile properties of PLA at 20 wt% loading, as no significant difference was found between their elongation at break or modulus when compared to nPLA ($p > 0.05$). Although structurally similar to ATBC, the branched glycerol analogs contain two additional ester groups and overall longer carbon chains to allow them to be both miscible with the polar PLA backbone, but also increase the free volume of the polymer matrix to effectively plasticize the blend.[18]

Overall, the glycerol analogs functioned to effectively plasticize PLA at both 10 and 20 wt% loadings and produce highly flexible and ductile solvent-cast films reaching elongations of up to 435%. In comparison, the melt-mixed blends plasticized with 10 wt% of the glycerol compounds showed negligible improvements in tensile properties relative to nPLA, while at 20 wt% loadings the plasticized blends showed significant improvement in ductility/flexibility with elongations up to 257% reached. The stark difference observed in the tensile properties at 10 wt% plasticizer loadings between the solvent-cast films and the melt-mixed blends is attributed to the size effect,[45, 46] in which thinner specimens demonstrate higher elongation than their thicker counterparts comprised of the same microstructure. This demonstrates the ability of these compounds to produce both highly flexible PLA films as well as bulk specimens while establishing their potential applicability for a variety of PLA-based food packaging materials.

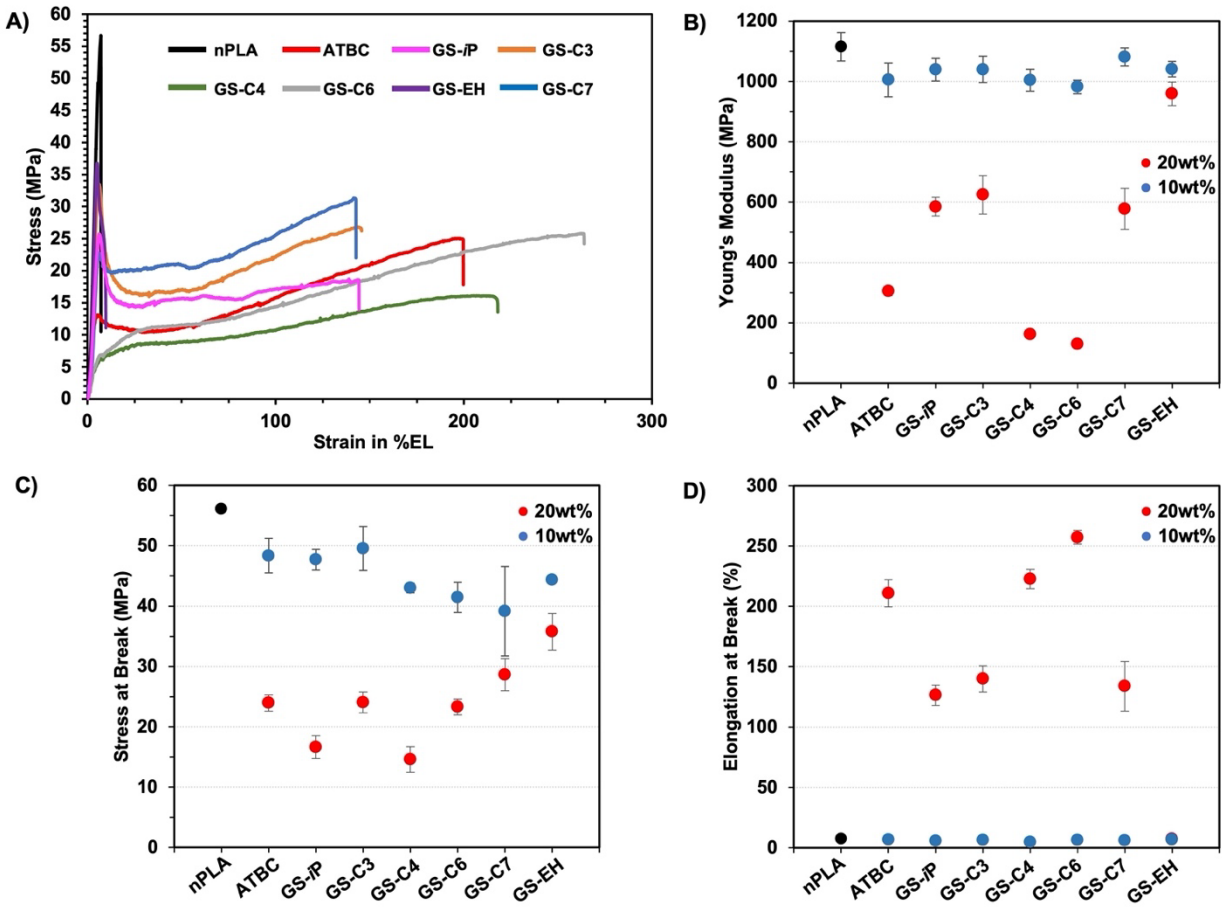

Figure 4. Mechanical properties of PLA melt-mixed blends at 20wt% plasticizer loadings: (A) Representative stress vs. strain in % elongation curves; (B) Young's modulus; (C) Stress at break; (D) Elongation at break (n=5, error bars represent standard deviation, means are shown).

Morphology of Blends

SEM of the freeze-fractured surfaces was used to characterize the morphology of both the solvent casted film blends (Figure 5) and the melt-mixed blends (Figure 6) at 20 wt% loadings of the plasticizers. The fractured surfaces of the films produced with ATBC, GS-C3, GS-*i*P, and GS-C4 all show relatively uniform incorporation of the plasticizer within the PLA matrix, which is demonstrated by a homogenous surface absent of any droplet formation or apparent phase separation. However, when observing blends produced with GS-C6, GS-EH, and GS-C7, an apparent phase separation occurs, which is evident by the formation of droplets and/or pores within the PLA matrix. Despite the presence of droplets and/or pores within the matrix, these three blends still showed significant improvements in tensile and thermal properties relative to nPLA.

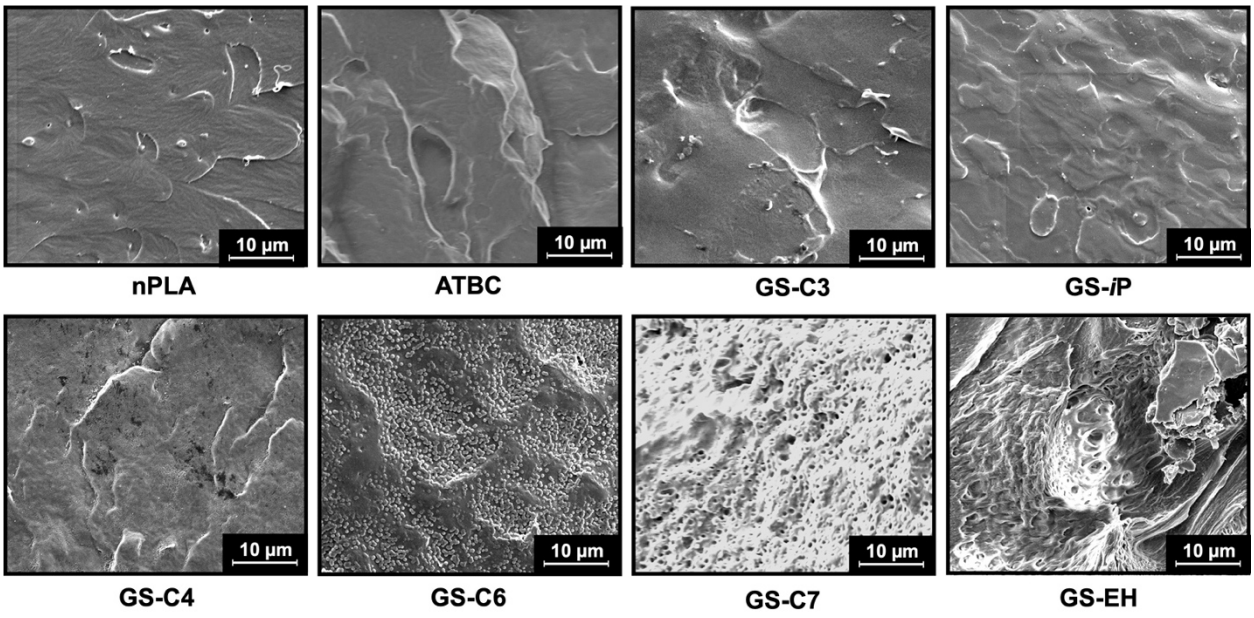

Figure 5. Freeze fractured surface SEM images of nPLA and 20 wt% solvent-cast film blends (2500× magnification).

In contrast, SEM images taken of the melt-mixed samples revealed different surface morphologies for several of the blends. While smooth, homogenous surfaces were observed in films produced with GS-*i*P and GS-C4, the melt-mixed blends show signs of droplet formation and potential phase separation. In the case of GS-C6, there was no sign of droplet formation in the melt-mixed blend and instead, a homogenous surface was observed. This observation of a well-compatibilized blend agrees with the remarkable tensile results obtained for GS-C6-20. Both preparation techniques yielded homogeneous morphologies with ATBC and GS-*i*P. The highly porous surface morphology obtained with GS-EH-20 is interesting as this unique architecture could be exploited for applications which require porous PLA materials, such as bone scaffolds.[47]

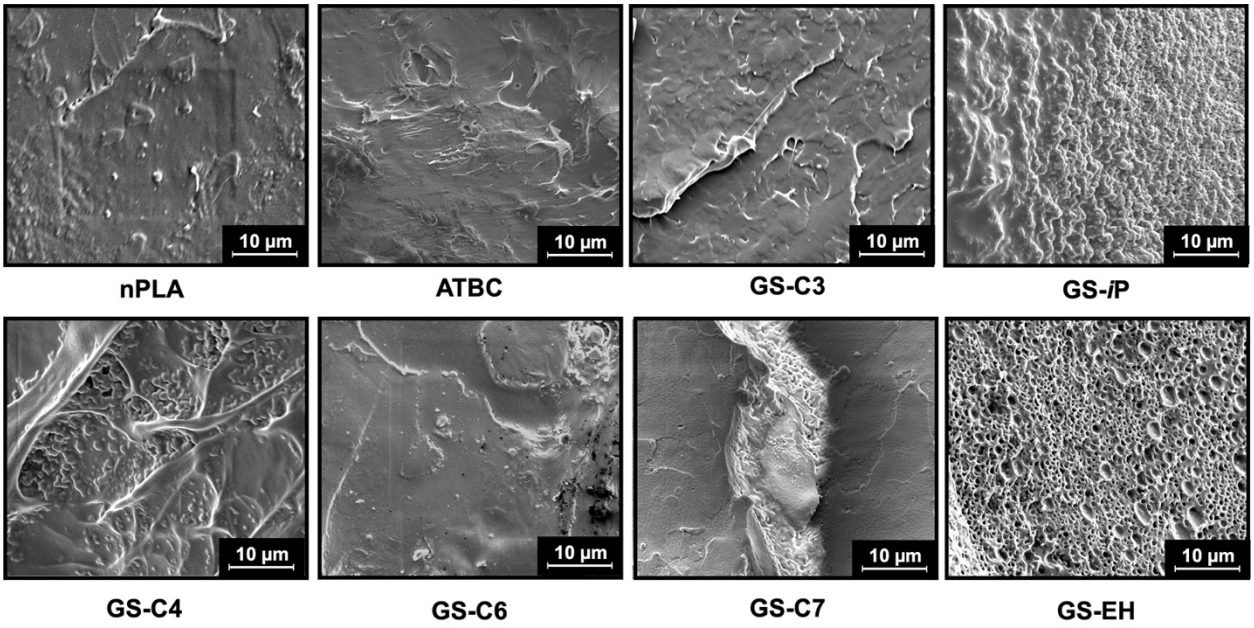

Figure 6. Freeze fractured surface SEM images of nPLA and 20 wt% melt-mixed blends (2500× magnification).

Plasticizer Leaching

The migration levels of each blend at 20 wt% were evaluated into three different food simulants to evaluate the applicability of each compound as potential plasticizers in food packaging material. Films were exposed to water, 3% v/v acetic acid, and 10% v/v ethanol for durations of one, five, and ten days at a temperature of 60 °C to monitor the evolution of migration over time (Figure 7). Significant differences amongst the blends were observed across all three simulants tested ($p < 0.001$). Previous studies have shown that lower molecular weight, more hydrophilic plasticizers generate higher degrees of migration out of PLA blends over time.[28, 48] A similar relationship was observed in our case as the blends produced with plasticizers comprised of longer alkyl chains exhibited the lowest percentage of mass loss over time. This effect of alkyl chain length was apparent amongst the plasticizers examined as GS-*i*P, functionalized with an *iso*-propyl chain, consistently displayed a higher mass loss after ten days than all other analogs tested ($p < 0.05$). In contrast, the blends produced with plasticizers functionalized with longer hexyl (GS-C6) and heptyl (GS-C7) alkyl chains displayed the lowest mass losses into all three simulants amongst the glycerol analogs, while ATBC exhibited the lowest mass loss overall.

Mass loss of the nPLA films remained relatively constant between 5-8% over time into all three simulants tested. This is attributed to the hydrolytic cleavage degradation of the ester bonds of the polymer into smaller oligomeric fragments.[49] Interestingly, blends produced with ATBC and GS-C6 exhibited a lower mass loss than nPLA into all three simulants examined ($p < 0.05$), suggesting that these plasticizers provide an added stability to the PLA matrix when exposed to aqueous solutions.

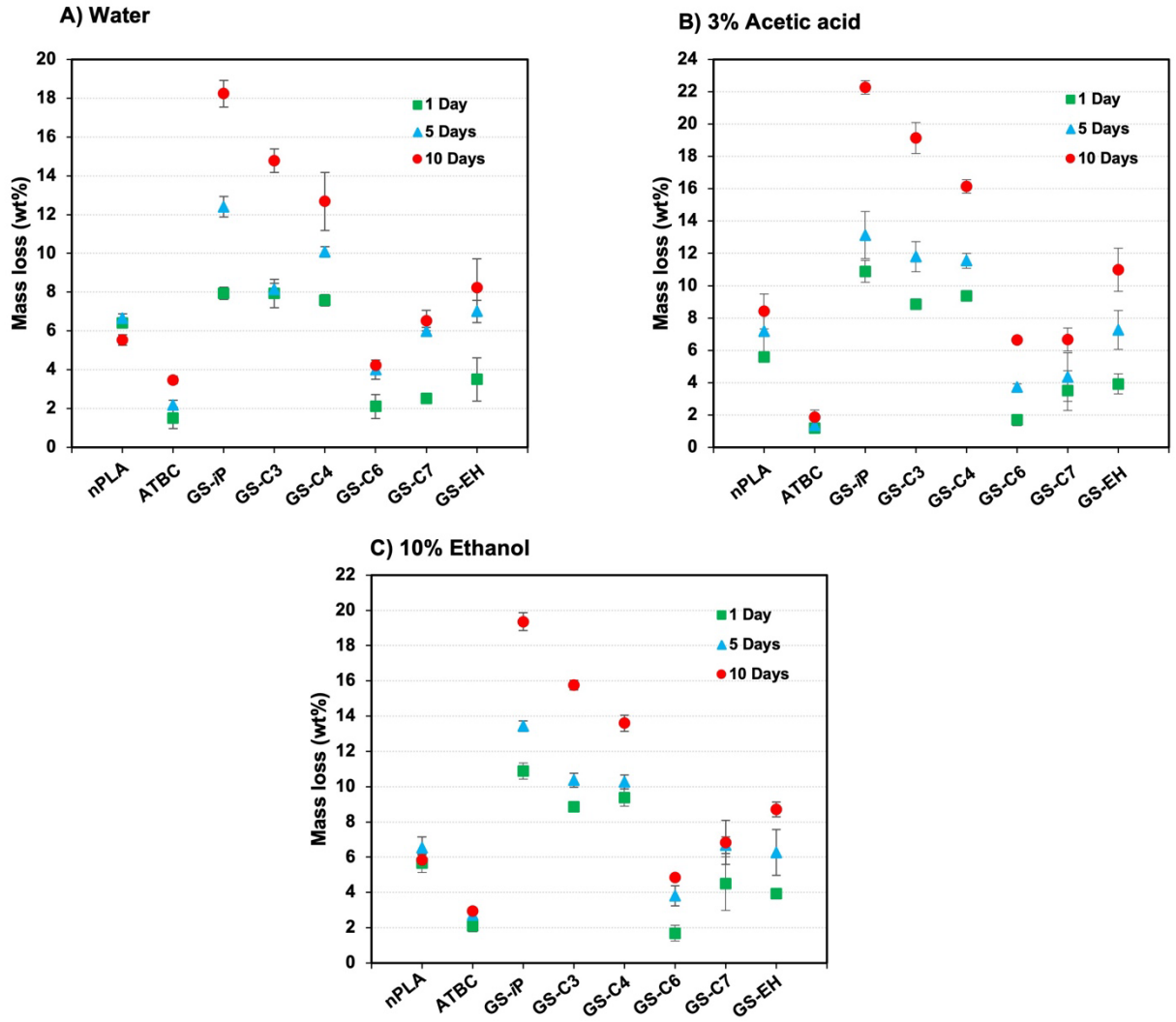

Figure 7. Mass loss due to leaching of nPLA and 20 wt% plasticized PLA film blends for durations of 1, 5, and 10 days in (A) water, (B) 3% v/v aqueous acetic acid, and (C) 10% v/v aqueous ethanol. (n=3, error bars represent standard deviation, means are shown).

Cell Viability Assay

While there are a number of promising alternative plasticizers for PLA being developed and reported in the literature,[50] the majority of these reports focus solely on the mechanical, thermal, and migration behaviour of the blends being produced and often overlook the evaluation of toxicity. The few examples which do include this type of analysis are essential in our progress towards developing all-encompassing green and sustainable plasticizers while avoiding regrettable substitution.[33] In line with this, we screened our new family of glycerol plasticizers for their cytotoxicity using an *in vitro* WST-8 cell viability assay[51] with HepG2 cells. The WST-8 reagent produces a formazan dye upon reduction by a metabolically active cell to allow for a direct quantification of viable cells and analysis of cytotoxicity. Plasticizers were administered to HepG2 cells at a concentration of 500 μM in 0.5% v/v DMSO (i.e., to enhance solubility in cell media) and absorbance readings were taken at time points of one and seven days. Despite the slight decrease in absorbance values after one day exposure to the plasticizers (Figure 8), there was no significant difference found between any of the plasticizers and the 0.5% v/v DMSO control ($p > 0.05$). However, after seven days exposure time, there was a significant difference found amongst the plasticizers tested ($p < 0.001$). Specifically, the absorbance values at day seven for GS-C7 and GS-EH were found to be significantly different than all the other plasticizers tested ($p < 0.05$), indicating a higher toxicity of these two longer chained or branched analogs. In contrast, there was no significant difference found between the shorter chain analogs (C6 or less) or ATBC with the 0.5% v/v DMSO control after seven days of exposure ($p > 0.05$). With GS-*i*P, GS-C3, GS-C4, and ATBC, the absorbance values increased between one and seven days which indicates that the cells were able to continue to proliferate in the presence of these compounds. Taken together, the results from this assay establish that the glycerol analogs comprised of alkyl chains equal to or shorter than C6 did not affect cell viability, whereas the longer chain or branched compounds demonstrated low to moderate levels of cytotoxicity and cell death. Follow-up work to investigate the biodegradation of the parent compounds, the identification of metabolites, and evaluation of their toxicity is proposed for future study.

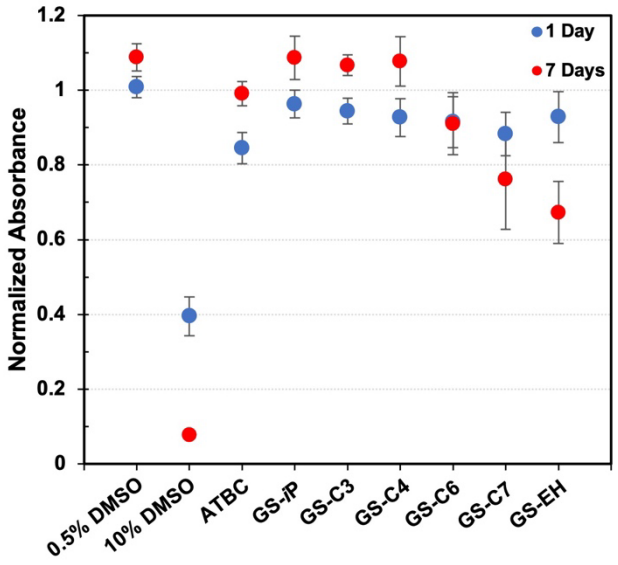

Figure 8. Normalized absorbance of HepG2 cells with and without the addition of plasticizers at 500 µM. 0.5% v/v DMSO was used as negative control while 10% v/v DMSO was used a positive control (n=3, error bars represent standard deviation, means are shown).

Conclusions

A family of bio-based plasticizers were designed and synthesized using solvent-free reaction conditions to produce flexible PLA which has potential applications as food packaging materials. Blends at 10 and 20 wt% plasticizer loadings were prepared using both solvent-casting and melt-mixing and analyzed to evaluate the effect of alkyl capper chain length and branching on plasticization efficiency and compared to blends produced using ATBC. In general, both the film and melt-mixed blends displayed higher thermal stability than blends prepared with ATBC. All glycerol analogs significantly reduced the T_g of nPLA, with the longer, linear substituted analogs providing the highest decrease in T_g of 44 °C relative to nPLA. The blends had excellent thermal stabilities with no significant decomposition observed below 208 °C at 20 wt% plasticizer loadings. Elongation at break values of up to 435% at 20 wt% plasticizer loadings were obtained in solvent-cast films, while the melt-mixed bulk samples reached elongation at break values up to 257%. The surface morphologies of the solvent-cast films and melt-mixed samples showed relatively smooth, homogenous mixtures for blends produced with plasticizers comprised of linear alkyl chains six carbons or smaller, whereas highly porous morphologies were obtained with branched or longer carbon chains. When exposed to different aqueous food simulants, film blends

plasticized with shorter alkyl chain groups showed the highest degree of migration over time with up to 22% mass loss observed after ten days, while GS-C6 blends displayed excellent migration resistance (<7% mass loss). Finally, the glycerol plasticizers comprised of alkyl chains six carbons or less were shown to be non-toxic through an *in vitro* mammalian cell toxicity assay. Taken together, this work demonstrates the applicability of this family of bio-plasticizers to produce highly flexible, low leaching, and non-toxic PLA blends which have the potential to be used to manufacture PLA-based food packaging materials.

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Supporting Information

Synthesis details and characterization, TGA, DSC, mechanical properties, solubility parameter calculations.

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