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**Reduction of poly(hydroxybutyrate-co-hydroxyvalerate) secondary crystallisation
through blending with saccharides**

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Abstract

A significant problem associated with the use of polyhydroxybutyrate and its copolymers in food packaging is their high initial crystallinity coupled with an ability to undergo secondary crystallisation over time resulting in progressive embrittlement. This paper details work undertaken to hinder the secondary crystallisation process through the blending of poly(hydroxybutyrate-co-hydroxyvalerate) (PHB-co-HV) with mono-, di- and tri-saccharides. Following blending, the melt viscosity and melting point were observed to decrease with increasing saccharide concentration. The incorporation of saccharides into PHB-co-HV did not completely prevent the secondary crystallisation process, however, the rate of change in the Young's modulus and ultimate tensile stress were significantly reduced. The percentage change in the mechanical properties of these blends was found to be directly related to the size of the saccharide which was deemed to be as a result of greater steric hindrance and an increasing number of hydroxyl groups available for interaction with PHB-co-HV. This, along with the reduction in melt viscosity and melting point, makes the addition of mono, di and tri saccharides worthwhile for improving the processability and prolonging the effective life time of products made from PHB-co-HV.

Keywords: poly(hydroxybutyrate-co-hydroxyvalerate); saccharide; secondary crystallization; blending; biodegradable; packaging

1. Introduction

Poly(hydroxybutyrate) and its respective copolymers are a series of biodegradable polymers [1-3] that have received considerable interest from the packaging industry as a result of barrier properties comparable to polyethylene terephthalate (PET) and polypropylene (PP).[4,

5] These polymers are formed through bacterial synthesis[6] and as a result have a high stereo specificity leading to crystallinities of 50-80%.[7, 8] Whilst this high crystallinity gives the aforementioned properties it also results in a brittle material. In addition, the sub-ambient glass transition temperature (approximately 4 °C)[7] of these copolymers allows secondary crystallisation to take place at room temperature via thickening of the lamellae and/or creation of new thinner lamellae.[9, 10] This leads to progressive embrittlement of the material and a reduction in ductility. The widespread use of PHB based polymers is also limited by a narrow processing window. High temperatures in excess of 180 °C are required to melt the polymers however, degradation can also occur at these temperatures leading to a reduction in the molecular weight and a deterioration in the mechanical properties of the material.[11, 12]

It has been established that intermolecular hydrogen bonding is a successful means of improving the mechanical and thermal properties of PHB and its copolymers.[13-16] Molecules such as Bisphenyl A (BPA) contain hydroxyl groups capable of hydrogen bonding to the carbonyl groups of PHB. These groups are separated by two bulky phenyl rings which limit the approach of the polymer chains and as a result primary and secondary crystallisation from the melt is hindered.[17] BPA has previously been reported to significantly reduce the crystallinity and improve the mechanical properties of PHB-co-HV[17-19] however, it is toxic to humans and therefore cannot be incorporated into food packaging.[20]

Starch displays similar functional groups to BPA but is non-toxic in the body. Previous attempts to blend starch with PHB has resulted in a reduction in crystallinity[21] coupled with enhanced thermal stability.[22] However, the high melting point of starch limited the potential for miscibility to occur on blending (the blending processing was carried out below

the melting point of the starch) and granules (approximately 10 μm) were observed by SEM.[21, 22] This microstructure caused a deterioration of the mechanical properties with reductions in the tensile strength and elongation to break being reported.[21]

As starch is a relatively long chain molecule consisting of numerous glucose units, the incorporation of smaller saccharides with melting points below that of PHB may offer potential as a property modifier. Fructose and glucose are 5 and 6 membered ring monosaccharides, respectively; whereas maltose and melezitose are di- and tri- saccharides consisting of glucose and fructose units (Figure 1). Each saccharide contains multiple alcohol groups which are capable of hydrogen bonding with the carbonyl groups of PHB. To date, there have been no reports in the literature on the blending of PHB and its copolymers with low molecular weight saccharides.

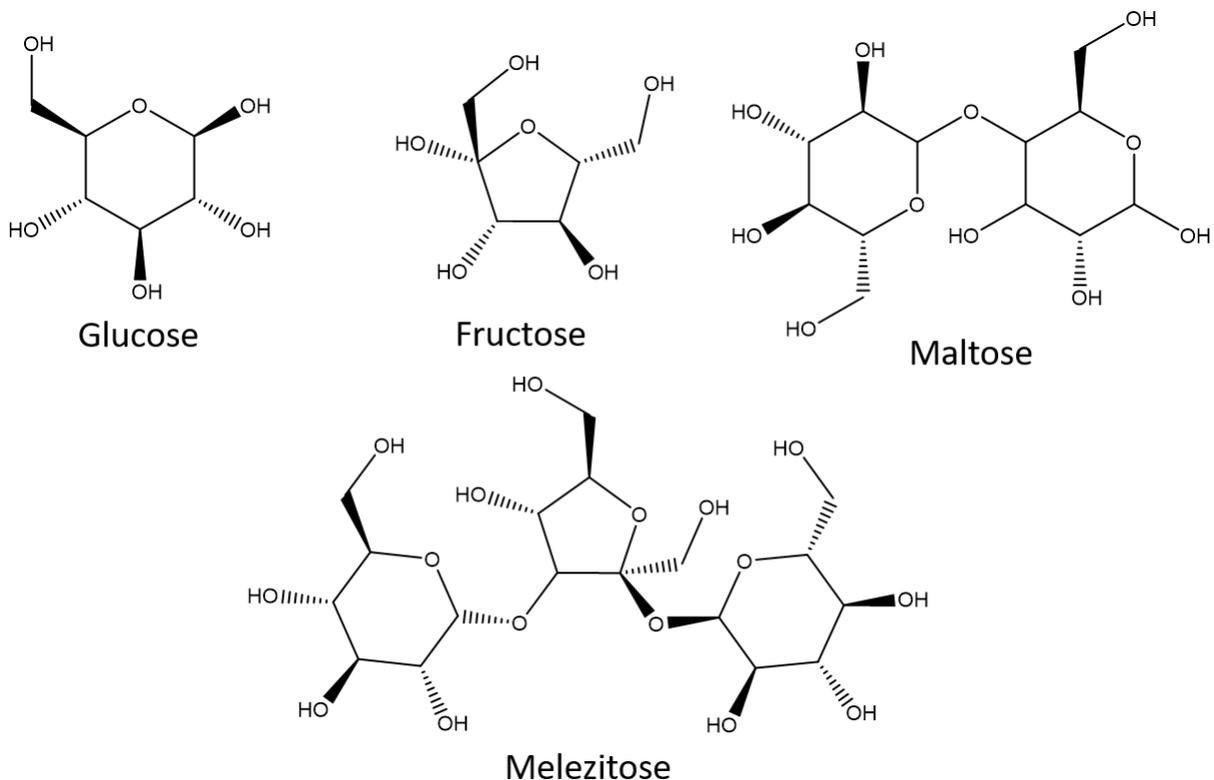


Figure 1: Chemical structures of glucose, fructose, maltose and melezitose

The rationale for this study is informed in part by the effectiveness of poly(ethylene glycol) (PEG) to successfully blend with PHB-co-HV resulting in hindrance of the secondary crystallisation process.[23, 24] As the saccharides shown in figure 1 have similar functional groups to PEG it is envisaged that they will also hinder the secondary crystallisation process. Therefore, blends of PHB-co-HV with various concentrations of fructose, glucose, maltose and melezitose are produced for the first time and the thermal and mechanical properties of each blend system analysed. These properties are also monitored over time to determine the effect of saccharides on the long-term secondary crystallisation of PHB-co-HV.

2. Material and Methods

2.1. Materials

Poly(hydroxybutyrate-co-hydroxyvalerate) (PHB-co-HV) containing 3% hydroxyvalerate (Tianan ENMAT Y1000P) was obtained from Helian Polymers (Venlo, Netherlands) and dried at 100 °C for 1 hour prior to use. The molecular weight has previously been determined as 215 kDa by gel permeation chromatography in chloroform using polystyrene standards.[25] D-(+)-Glucose, D-(-)-Fructose, D-(+)-Maltose monohydrate and D-(+)-Melezitose monohydrate were purchased from Sigma Aldrich (Dorset, UK) and used as received. Nitrogen (O₂ free) was purchased from BOC gases (Guildford, UK).

2.2. Methods

2.2.1. Melt Blending

PHB-co-HV was blended with fructose, glucose, maltose and melezitose in various concentrations using a Haake PolyLab QC mechanical mixer (ThermoScientific,

Loughborough, UK). The mixer was preheated to 170 °C and the required mass of saccharide stirred into PHB-co-HV by hand before adding it (in 50 g portions) to the Haake mixer. The materials were mixed for 10 minutes at a screw speed of 100 rpm before the blend was collected and stored in a freezer (-22 °C) until required.

2.2.2. Control of Blend Composition

As the saccharides are expected to reduce the secondary crystallisation of PHB-co-HV by creating steric hindrance one focus of this study is to assess the effect of saccharide size. Therefore it is important that the number of molecules and therefore the mole ratio of saccharide to PHB-co-HV repeat unit remains constant throughout. The mole ratios were selected to match those previously used in the blending of PHB-co-HV and PEG[23] and are denoted as low, medium and high saccharide contents (Table 1).

Table 1: Mole ratio of saccharide to PHB-co-HV repeat unit and %w/w additive required for each saccharide concentration

| Sugar | Saccharide content | Saccharide : PHB-co-HV mole ratio | %w/w of additive |
|--------------|---------------------------|--|-------------------------|
| Glucose | Low | 0.011 | 2.24 |
| | Medium | 0.024 | 4.76 |
| | High | 0.038 | 7.33 |
| Fructose | Low | 0.011 | 2.24 |
| | Medium | 0.024 | 4.76 |
| | High | 0.038 | 7.33 |
| Maltose | Low | 0.011 | 4.17 |
| | Medium | 0.024 | 8.67 |
| | High | 0.038 | 13.1 |
| Melezitose | Low | 0.011 | 6.03 |
| | Medium | 0.024 | 12.3 |
| | High | 0.038 | 18.1 |

The mass of saccharide required (Table 1) was calculated using the equation below. As hydroxybutyrate has a mass of 86 and hydroxyvalerate a mass of 100 this gives an average repeat unit mass of 86.42 for the copolymer containing 3% valerate.

$$\text{Saccharide (\%wt)} = \frac{\text{moles}_{\text{saccharide}} \times M_w \text{ saccharide}}{(\text{moles}_{\text{saccharide}} \times M_w \text{ saccharide}) + (\text{moles}_{\text{PHB-co-HV unit}} \times M_w \text{ PHB-co-HV unit})} \times 100\%$$

2.2.3. Hot Pressing

Plaques (152 x 158 x 0.266 mm) of the PHB-co-HV : saccharide blends were produced by compression moulding using a Moore E1127 hydraulic hot press (George E. Moore & Sons Ltd, Birmingham, UK). The blend (9 g) was placed into a mould (152 x 158 x 0.266 mm) which was then inserted into the press at 180 °C. The blend was allowed to warm for 3 minutes before a load of 10 tonnes was applied for a further 5 minutes. The plaques were cooled for 20 minutes by passing tap water (24 °C) through the plates giving a cooling rate of approximately 17 °C min⁻¹. The plaques were immediately cut into the required shapes and stored in a freezer (-22 °C) until required to prevent further crystallisation. For the rheological analyses, plaques of 77 x 77 x 1 mm were produced using 8 g of the polymer blend.

2.2.4. Stability Study

In order to determine the effect of various saccharides on the rate of secondary crystallisation and embrittlement, time studies were performed. The samples were removed from the freezer and allowed to equilibrate to room temperature for 10 minutes. The day 0 samples were analysed immediately by differential scanning calorimetry (DSC) and mechanical

testing and the remainder stored at room temperature and analysed at the following time points; 1, 2, 3, 4, 8, 12, 24, 38 and 48 weeks.

2.2.5. Rheology

The viscosity of the polymer blends was measured using a Rheometric Scientific ARES rheometer (Surrey, UK) with a 25 mm parallel plate geometry. Discs, 25 mm in diameter, were cut from the 1 mm hot pressed plaques and inserted into the rheometer. The rheometer was heated to 180 °C and the samples left for 5 minutes to ensure complete melting. Oscillation tests were performed, at a frequency of 10 Hz, in which the shear strain was increased logarithmically from 1 to 350 %. Three samples were analysed for each blend and the average and standard deviation calculated.

2.2.6. Differential Scanning Calorimetry (DSC)

The melting points and crystallinities of the PHB-co-HV blends were measured using a Mettler Toledo DSC 1/500 (Mettler Toledo, Leicester, UK). The instrument was calibrated with indium, tin and zinc standards. Circular samples (~5 mg) were punched from the hot pressed plaques and weighed into standard 40 µl aluminium DSC pans (Mettler Toledo). The pan was capped with an aluminium DSC lid (Mettler Toledo) and sealed with a press (Mettler Toledo) before inserting into the DSC at room temperature and cooling to -40 °C. The melting point was taken at the peak of the transition when heating from -40 to 200 °C at a rate of 10 °C min⁻¹. The crystallinity of PHB-co-HV in the blends was determined by heating to 220 °C, at a rate of 50 °C min⁻¹ following a hold at -40 °C for 5 minutes. A linear baseline was drawn from the first onset of melting to the last trace of crystallinity and the enthalpy of

fusion was calculated from the area under the endotherm. The degree of crystallinity (X_c) of the PHB-co-HV fraction is defined as:

$$X_c = \frac{\Delta H_f}{\Delta H_f^0} \times \frac{1}{M_{PHB-co-HV}} \times 100\%$$

Where ΔH_f is the enthalpy of fusion of the blend; ΔH_f^0 is the enthalpy of fusion of completely crystalline PHB-co-HV and $M_{PHB-co-HV}$ is the mass fraction of PHB-co-HV in the blend.

A literature value of 146 J g^{-1} was used for the ΔH_f^0 of PHB-co-HV.[26] Thermal analysis was limited to the first heating run to ensure that the observations were related to the initial processing only.

2.2.7. Mechanical Testing

The Young's modulus, ultimate tensile strength (UTS) and elongation to break were determined using an Instron 5566 (High Wycombe, UK) controlled by Merlin software. Samples, in the form of dog bones (13 x 75 mm), were cut from the hot-pressed plaques and extended at a rate of 2 mm min^{-1} . Loads were recorded using a 10 kN load cell and stress-strain data was recorded at 500 ms intervals. Three samples were analysed for each time point and the average and standard deviation calculated.

2.2.8. Statistical Analysis

A student t-test was performed at the 95 % confidence level on the data. The results were considered significant if the calculated p-value was less than 0.05.

3. Results and Discussion

3.1. Effect of the Addition of Saccharides on the Initial Properties of PHB-co-HV

Each saccharide was incorporated in three different concentrations and the resulting blends hot pressed into plaques. To facilitate discussion, the saccharide concentrations are denoted as ‘low,’ ‘medium’ and ‘high’ in this work and equate to saccharide : PHB-co-HV repeat unit mole ratios of 0.011, 0.024 and 0.038, respectively (Table 1). Processing of pure P(HB-co-HV) produced a milky white material, however addition of the saccharides rendered the samples various shades of brown (Figure 2). This colour change is indicative of either degradation of the polymer or caramelisation of the saccharide. As the samples were all processed under the same conditions, and the unblended material remains unaltered, the colour change was deemed to originate from caramelisation of the saccharides during processing.

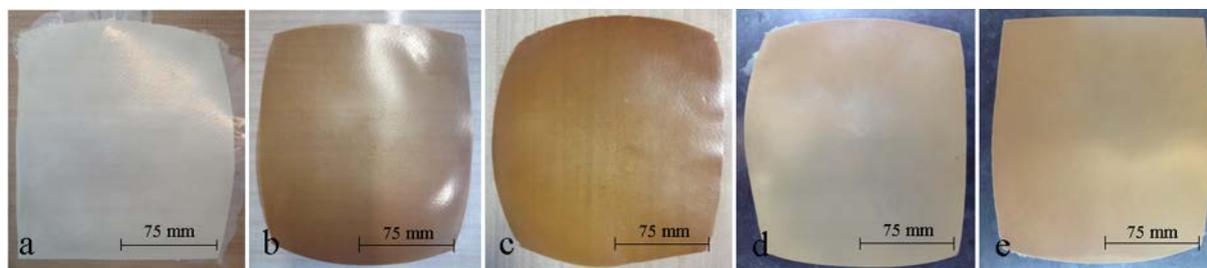


Figure 2: Photographs of plaques produced containing a) pure P(HB-co-HV) and low concentrations of b) glucose c) fructose d) maltose and e) melezitose

Saccharides are well known to caramelize on heating above a set temperature (110, 160 and 180 °C for fructose, glucose and maltose, respectively). The additives composed of larger units (maltose and melezitose) showed a reduced colour change compared to the monosaccharides despite a greater weight percentage in the blends. This is due to the presence of glycosidic bonds in maltose and melezitose making them more stable[27] and

therefore creating higher caramelisation temperatures.[28] Researchers have previously modelled the colour changes of fructose, glucose and sucrose during caramelisation as a means of determining the reaction rate.[29, 30] In all cases a time lag was observed followed by an exponential increase in browning. Luna et al. also monitored these colour changes at 170 °C which is comparable to the processing temperatures adopted in this work.[29] After 10 minutes the authors reported only slight colour changes for both glucose and fructose which gives confidence that the majority of the saccharides are still in their native form when incorporated into PHB-co-HV.

Rheological studies were performed on the blends to determine the effect of the saccharides on the viscosity, and therefore processability, of PHB-co-HV (Figure 3). Each of the samples exhibited Newtonian behaviour in the first portion of the trace (below 30 % strain) where viscosity was found to be independent of strain. As the strain increased, chain alignment and shear thinning occurred as expected creating a reduction in the viscosity.[31]

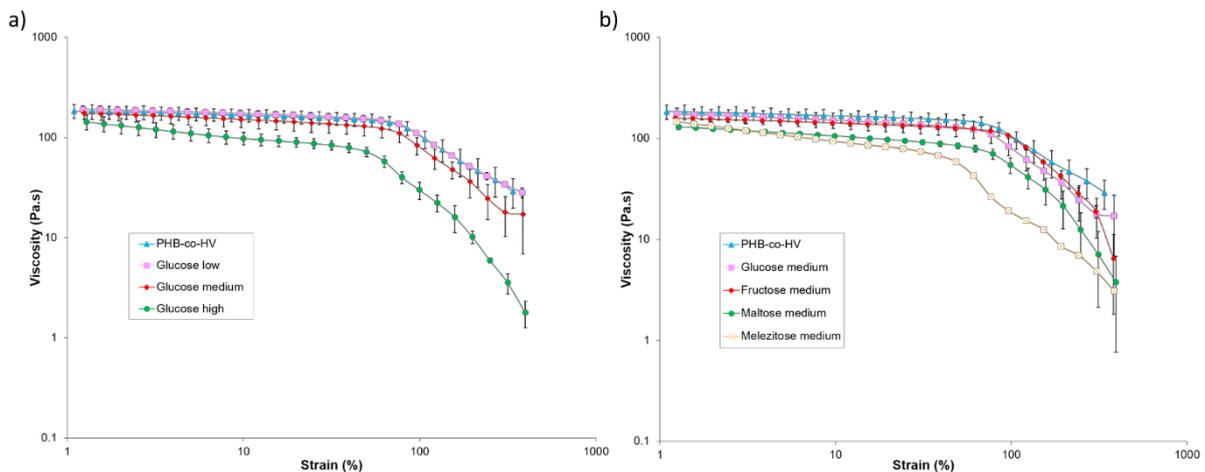


Figure 3: Viscosity analysis of PHB-co-HV : saccharide blends. Effect of a) increasing glucose concentration b) altering the size of the saccharide

The addition of a low content of glucose showed no variation in the viscosity of pure PHB-co-HV (Figure 3a), presumably the concentration being too low to yield any significant effects. However, as the concentration of glucose increased, the viscosity reduced; with a 35% reduction observed at a strain of 10% for the highest concentration. Similar trends were generally experienced across each of the saccharides analysed. A decrease in viscosity is indicative of the saccharides ability to plasticise PHB-co-HV. In the melt the saccharides occupy positions between the polymer chains disrupting the intermolecular interactions and increasing the free volume. This results in a lower resistance to flow compared to the pure polymer and therefore a reduced melt viscosity.[32] The effect is exaggerated as the saccharide content increases.

Comparison of the blends containing medium saccharide concentrations revealed little difference in the viscosities of the glucose and fructose containing blends, possibly due to the similarity in their size. However, on increasing the size to a disaccharide (maltose) or trisaccharide (melezitose) the viscosity significantly reduced. These larger molecules create greater inter-chain separation enhancing the plasticisation effect and thereby further reducing the viscosity.

Using DSC, a single endotherm was observed for each sample with no evidence of the corresponding melting points of the saccharide components. This suggests that the saccharides remain dispersed in either the inter-lamella or inter-spherulitic regions and is prevented from crystallising; the saccharide could be deemed to be miscible with the amorphous phase of PHB-co-HV. The presence of each saccharide induces a melting point depression proportional to the size, and to some extent, the concentration in the blend (Table 2). A reduction in the melting point is indicative of the formation of thinner lamellae and

supports the idea that following crystallisation of the PHB-co-HV component, the saccharides remain within the inter-lamella regions. In the melt, interactions may develop between the hydroxyl groups of the saccharides and the carbonyl group of the PHB-co-HV repeat units. On cooling, the PHB-co-HV component will begin to crystallise but the presence of the saccharide molecules hinders this process and limits the lamella thickness, depressing the melting temperature. Within experimental error (± 2 % crystallinity) this reduction in melting point is not accompanied by a change to the crystallinity. This suggests that a large number of thinner lamellae must be formed when saccharides are incorporated into PHB-co-HV. A similar reduction in the melting point of PHB-co-HV, accompanied by no change in the crystallinity, has been reported previously following blending with poly(ethylene glycol) in a range of molecular weights.[23]

Table 2: Initial thermal properties of the PHB-co-HV:saccharide blends. Experimental error in the: melting point ± 0.3 °C; crystallinity ± 2 %

| Additive Content | PHB-co-HV melting point (°C) | | | PHB-co-HV crystallinity (%) | | |
|---------------------|------------------------------|--------|------|-----------------------------|--------|------|
| | Low | Medium | High | Low | Medium | High |
| PHB-co-HV | | 171 | | | 62 | |
| Glucose | 168 | 166 | 164 | 63 | 60 | 56 |
| Fructose | 169 | 168 | 166 | 61 | 61 | 60 |
| Maltose | 167 | 167 | 167 | 62 | 64 | 58 |
| Melezitose | 164 | 162 | 163 | 58 | 62 | 65 |

PHB and its copolymers have a narrow processing window in that while temperatures in excess of 180 °C are required to process the polymer, significant degradation occurs just above the melting point.[11, 12] A melting point reduction is therefore beneficial as it allows the material to be processed at a lower temperature, increasing this window and reducing the likelihood of degradation.

No glass transition temperature could be observed in the DSC traces. This is commonly seen with PHB and its copolymers [23, 33] and has been attributed to a low amorphous content.

Following hot pressing, mechanical tests were performed to determine how the addition of saccharides affected the Young's modulus, ultimate tensile strength and elongation to break of PHB-co-HV (Figure 4). A student t-test performed on the results showed no significant change to the YM, UTS or, with the exception of melezitose ($p=0.047$), elongation to break on adding low concentrations of the saccharides to PHB-co-HV. This compliments the rheological data which demonstrates that low concentrations of the saccharides are unable to sufficiently weaken the intermolecular interactions of the polymer, increase the free volume and further mobilise the polymer chains.

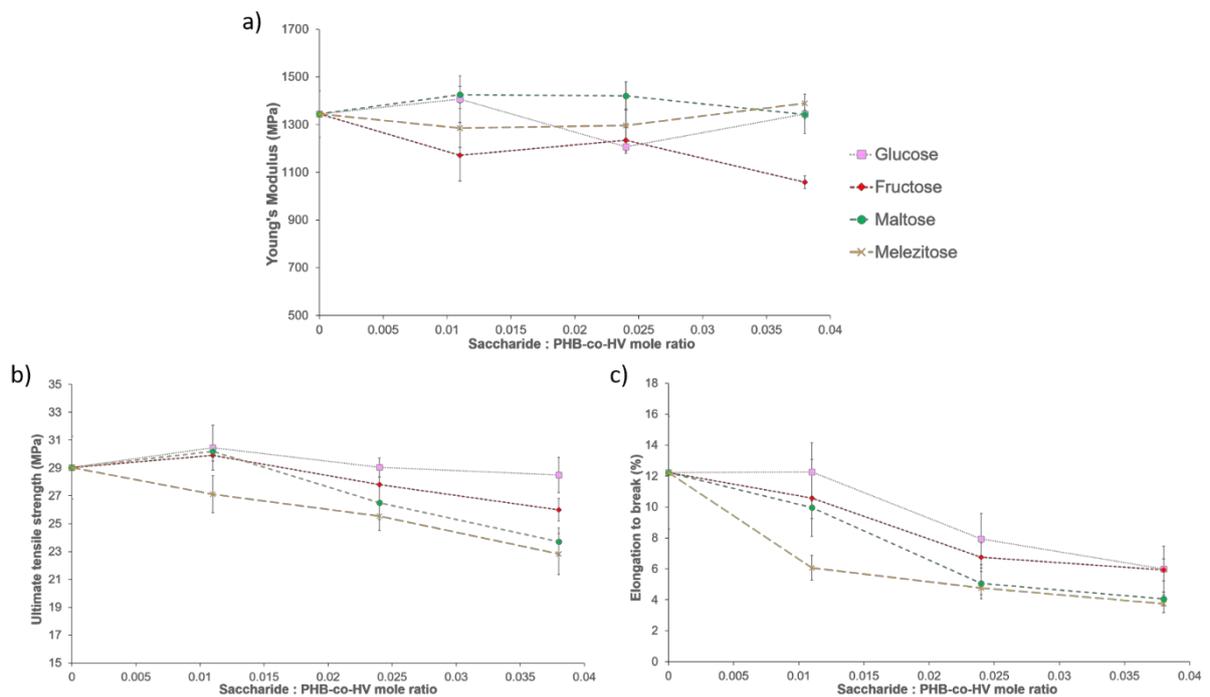


Figure 4: Initial mechanical properties of the PHB-co-HV : saccharide blends: a) Young's modulus; b) ultimate tensile strength and c) elongation to break. Dashed lines have been superimposed between the points as a visual aid to guide the eye through the data sets.

Only the highest concentration of fructose was found to significantly ($p=0.049$) lower the Young's modulus of PHB-co-HV (Figure 4a) whereas, high concentrations of maltose ($p=0.050$) and melezitose ($p=0.016$) significantly lowered the ultimate tensile strength (Figure 4b). The elongation to break was found to be dependent on both the concentration and size of the saccharide with the increase of either creating a less ductile material (Figure 4c). The reduction in elongation to break is believed to be as a result of the formation of more, smaller lamellae on crystallisation as illustrated by the DSC analysis. The increase in the number of lamellae will result in more rigid amorphous material and as a result the mobile amorphous fractions will reduce. This will decrease the amount of amorphous material able to align during tension and therefore the elongation to break.

Reductions in these mechanical properties are unfavourable as they reduce the structural properties of the material, however for the majority of the samples analysed, the blended material is comparable to the pure form. In addition, the reductions observed here are lower than those reported previously with PEG[23] indicating that the saccharides may prove to be a superior additive.

3.2. Hindrance of Secondary Crystallisation

The secondary crystallisation process occurs through an increase in lamella thickness[34, 35] and/or the formation of smaller subsidiary lamellae within the amorphous regions between

the original lamellar stacks.[34-36] This acts to increase the crystalline fraction of the polymer. The crystallinity of each of the blends and pure PHB-co-HV were analysed periodically over 28 days (data not shown) as previous studies have shown this to be when the crystallinity increase is most prevalent.[9, 10, 25] Student t-tests performed on day 0 and day 28 showed no significant increase in the crystallinity over time ($p>0.18$). A similar observation has been reported previously with PHB-co-HV and PEG blends.[23, 24]

The secondary crystallisation process is also known to be accompanied by an increase in Young's modulus and ultimate tensile strength and a decrease in elongation to break.[9, 10] The tensile properties of PHB-co-HV and the saccharide blends were monitored periodically over an extended time period of 48 weeks (Figures 5 and 6). The results show that the addition of the saccharides did not completely prevent the secondary crystallisation process: changes to mechanical properties were observed in each blend composition studied. For clarity only the results for fructose are displayed (Figure 5) however each of the saccharides displayed similar trends. The addition of low and medium amounts of fructose had little effect on the rate of secondary crystallisation, however a higher content hindered the process and slowed the increases of the Young's modulus (Figure 5a) and ultimate tensile strength (Figure 5b). The decrease in elongation to break occurred at a similar rate for each concentration analysed and was similar to that of pure PHB-co-HV (Figure 5c). As discussed previously, on cooling the saccharides are rejected from the crystalline growth fronts and expelled to the inter-spherulitic and inter-lamella regions. It is from within the inter-lamella regions where they act to hinder the secondary crystallisation process either by preventing the formation of new and relatively thin lamella in the mobile amorphous fraction or by hindering the re-organisation of the crystallisable chains located in the rigid amorphous

fraction. Accordingly, as the concentration of fructose within these blends increases, the effect becomes more pronounced.

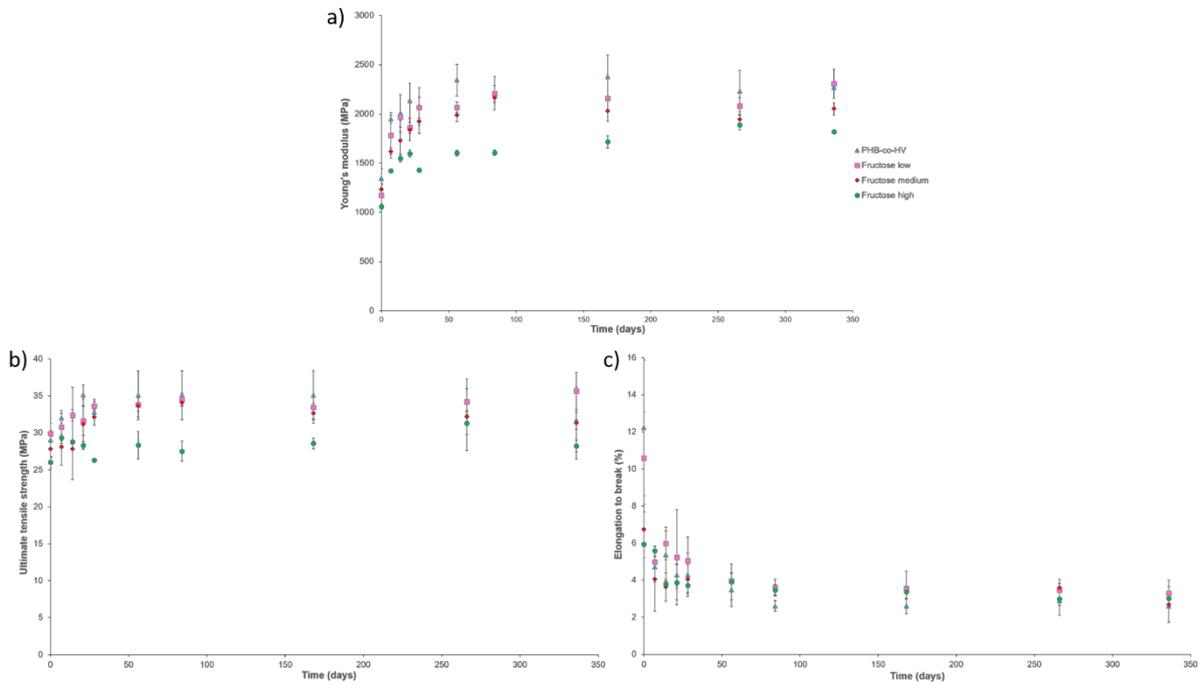


Figure 5: Effect of the addition of fructose on the a) Young's modulus: b) UTS and c) elongation to break over time compared to pure PHB-co-HV.

The effect of the different saccharides on the rate of secondary crystallisation was also analysed and the results for the concentrations denoted as medium are shown in Figure 6. The majority of the saccharides appeared to follow the same trend as pure PHB-co-HV however melezitose was found to significantly limit the increase of both the Young's modulus (Figure 6a) and UTS (Figure 6b).

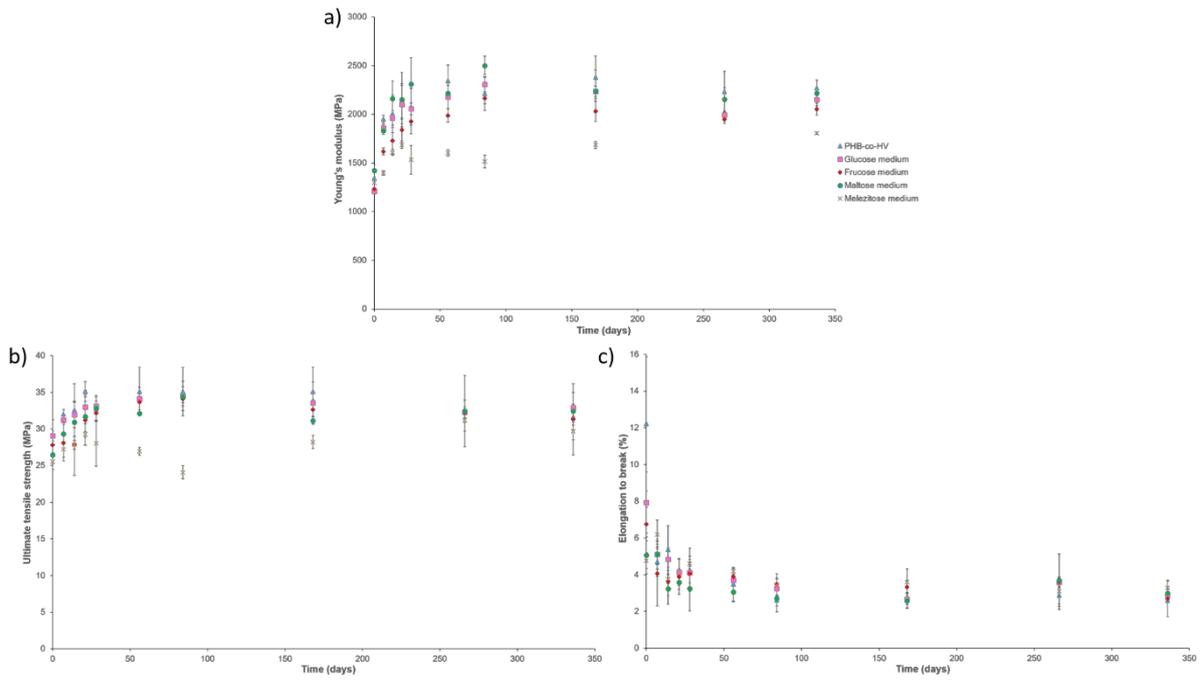


Figure 6: Effect of the addition of various saccharides on the a) Young's modulus b) UTS and c) elongation to break over time compared to pure PHB-co-HV

One thing to note is that the initial Young's modulus, ultimate tensile strength and elongation to break varied between the samples and therefore a more appropriate method to analyse the effect of the addition of saccharides is to evaluate the percentage change in each property (Figure 7).

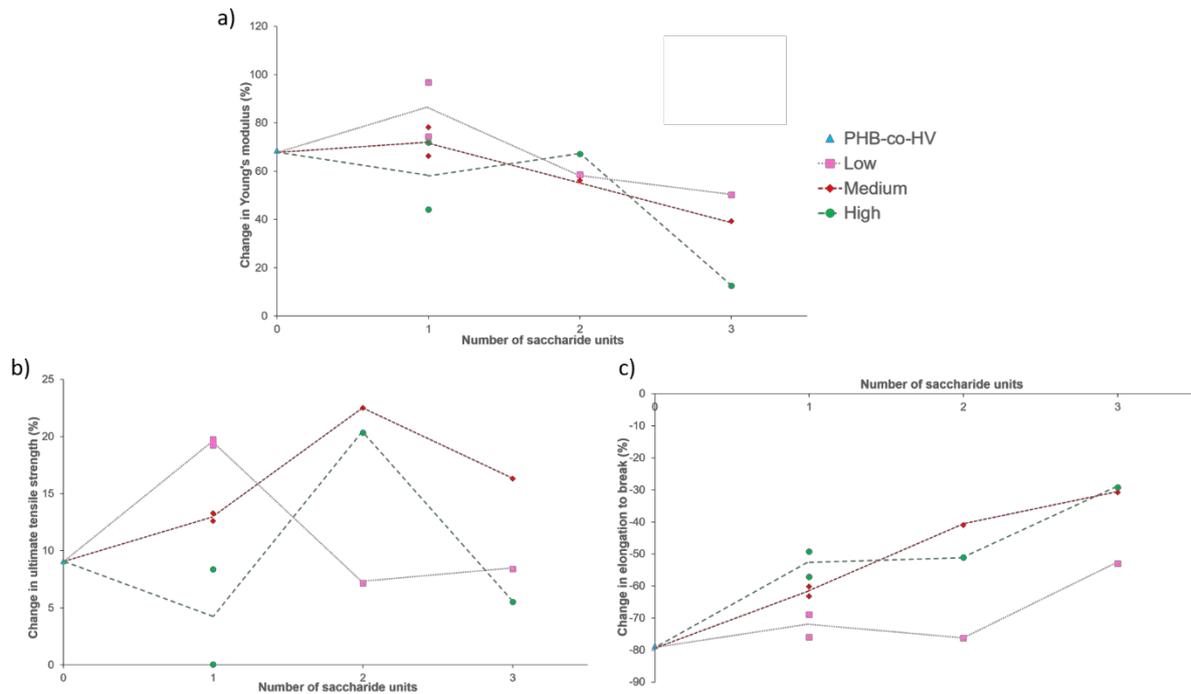


Figure 7: Effect of the saccharide chain length on the total percentage change in the a) Young's modulus b) UTS and c) elongation to break over time compared to pure PHB-co-HV. Dashed lines have been superimposed between the points as a visual aid to guide the eye through the data sets.

Analysis of the percentage change in the Young's modulus (Figure 7a) and elongation to break (Figure 7c) show that increasing the size of the saccharide, by adding more glucose/fructose units, significantly limited the change in both properties (and therefore the extent of secondary crystallisation). The bulkier structure created by increasing the number of saccharide units creates a larger steric hindrance between the polymer chains which impedes the rearrangement necessary for secondary crystallisation. In addition, the presence of more saccharide units will result in an increase in the number of hydroxyl groups available for interaction with the PHB-co-HV units. No direct relationship was found between the size of the saccharide and the change in the ultimate tensile strength.

Chen et. al have recently shown that during secondary crystallisation the increase in crystallinity is proportional to the square root of time.[37] These, and similar studies,[38, 39] were only performed over relatively short time scales of 20 hours and so the effect of long term storage on the applicability of the model has not yet been evaluated. As discussed above, the crystallinity of the samples displayed no significant increase over the time scale of the study however, changes were observed in the mechanical properties. The Young's modulus, ultimate tensile strength and elongation to break were plotted against the square root of time for each of the blends produced. No discernable trends were found in the ultimate tensile strength and elongation to break possibly due to the influence of external factors such as surface defects. As the Young's modulus is taken from the initial part of a stress/strain curve it provides the most accurate representation of the material.

A plot of the Young's modulus of PHB-co-HV against the square root of time displayed a linear relationship over the first 9 weeks after which a plateau was observed (Figure 8). This plateau is indicative of the completion of the secondary crystallisation process as no further change to the Young's modulus is observed. A similar trend was also found with the majority of the saccharides in low and medium concentrations however the secondary crystallisation process was found to continue for an additional 3 weeks when a similar final Young's modulus was observed. The increase in Young's modulus was found to be considerably slower in the high saccharide concentration samples with no plateau observed over the length of the experiment demonstrating a prolonged secondary crystallisation process. Additionally, the Young's modulus of the blends with high saccharide concentrations remained significantly lower following the 48 week study.

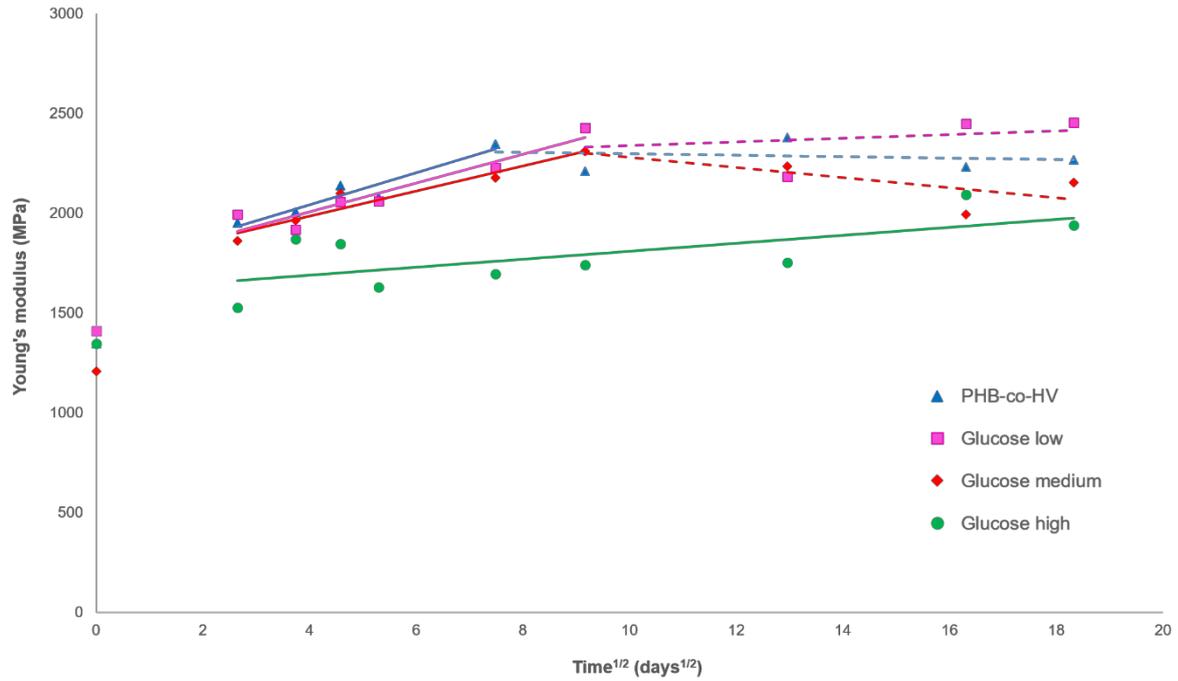


Figure 8: Evaluation of the secondary crystallisation kinetics of PHB-co-HV containing various concentrations of glucose. Solid lines indicate the secondary crystallisation process. Dashed lines display the negligible further changes to the Young's modulus i.e. the end of secondary crystallisation.

In previous work the relationship between secondary crystallisation and time has been given by the following equation[37-39]:

$$\frac{X_{s,t}}{X_{p,\infty}} = (1 + k_s t^{1/2})$$

Where $X_{s,t}$ is the crystallinity at time t , $X_{p,\infty}$ is the final fractional crystallinity at the end of the primary process and k_s is rate constant for secondary crystallisation. This can be modified to give:

$$Young's\ modulus = (c + k_s t^{1/2})$$

Where the secondary crystallisation rate constant can be determined from the gradient of the Young's modulus versus the square root of time plots.

The gradient of the points corresponding to the secondary crystallisation portions of the graphs (Figure 8) were calculated (Table 3). The decision was taken to analyse the entire secondary crystallisation process, rather than taking the same time period for each sample, to gain more accurate results. The gradient of the curves was then used to calculate the rate constants for each of the PHB-co-HV : saccharide blends along with the R^2 values (Table 3). In addition the duration of the secondary crystallisation process, t , is also reported.

Table 3: Secondary crystallisation rate constants for PHB-co-HV : saccharide blends

| Saccharide | PHB-co-HV | | | Low | | | Medium | | | High | | |
|------------|-------------------------------------|-------|----------|-------------------------------------|-------|----------|-------------------------------------|-------|----------|-------------------------------------|-------|----------|
| | K_s (MPa day ^{-1/2}) | R^2 | t (days) | K_s (MPa day ^{-1/2}) | R^2 | t (days) | K_s (MPa day ^{-1/2}) | R^2 | t (days) | K_s (MPa day ^{-1/2}) | R^2 | t (days) |
| Fructose | | | | 56.8 | 0.80 | 84 | 77.9 | 0.95 | 84 | 25.8 | 0.84 | 336 |
| Glucose | 80.3 | 0.91 | 56 | 71.9 | 0.90 | 84 | 62.7 | 0.93 | 84 | 20.0 | 0.45 | 336 |
| Maltose | | | | 85.8 | 0.96 | 84 | 77.3 | 0.72 | 84 | 27.7 | 0.57 | 336 |
| Melezitose | | | | 16.1 | 0.68 | 336 | 23.9 | 0.62 | 336 | 8.2 | 0.09 | 336 |

From table 3 it can be seen that PHB-co-HV and blends containing low and medium concentrations of the small saccharides display high R^2 values demonstrating the applicability of the model over extended timescales. In addition, the rate constants were found to reduce with saccharide concentration and size as a result of the steric hindrance created by the saccharides impeding the chain rearrangement needed for secondary crystallisation. This effect occurs more prominently at the high concentrations and with large saccharides where the calculated rate constant reduces further. However, in these cases low R^2 values are produced signifying an unsuitability of the model.

4. Conclusion

A concentration dependant reduction in the viscosity of PHB-co-HV was observed on the addition of a variety of saccharides which is indicative of successful blending in the melt. In the majority of cases the saccharides produced no significant detrimental effects on the mechanical properties of PHB-co-HV. Despite the addition of the saccharides, secondary crystallisation was still found to occur, however, the extent to which this occurred was greatly reduced. The size of the saccharide was found to directly influence the percentage change in the Young's modulus and elongation to break with the tri-saccharide, melezitose, hindering the secondary crystallisation process to the greatest extent. As these blends are able to reduce the rate of secondary crystallisation, without a significant loss to the initial mechanical properties, they provide an effective means to extend the shelf life of the packaging material. In addition, the observed reduction in the melting point extends the processing window and thereby reduces the chance of polymer degradation.

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