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Jenkins, Michael; Kelly, Catherine

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Secondary crystallisation and degradation in P(3HB-co-3HV): an assessment of

long-term stability

Running head: P(3HB-co-3HV) long-term thermal stability

Mike J. Jenkins¹, Kate E. Robbins¹ & Catherine A. Kelly^{1*}

School of Metallurgy and Materials, University of Birmingham, Edgbaston, Birmingham,

B15 2TT, UK

*to whom correspondence should be sent: email - c.a.kelly@bham.ac.uk; telephone number - 0121 414 3436

Abstract

The effect of long-term storage, at a range of temperatures, on the thermal and mechanical properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) is reported. P(3HB-co-3HV) was stored at -22, 7, 20, 50, 75, 100, 125 and 150 °C for 672 hours. Below 100 °C, the observed increase in melting point, glass transition temperature, modulus and strength are due to the development of crystallinity via a secondary process and facilitated by increasing temperature. At 125 °C, the processes of secondary crystallisation and degradation occur in parallel and there is an interplay between these processes, with the effects of degradation masked by the continuation of secondary crystallisation. At 150 °C there is a deterioration in all the properties noted above and given the reduction in molecular weight (and discolouration), this can be attributed to degradation. The significant observation from these results is that the degradation process remains active well below the melting point and long-term stability of P(3HB-co-3HV) is not only affected by secondary crystallisation, but also degradation. In addition, as no significant changes to the thermal or mechanical properties were observed during storage at -22 and 7 °C it suggests that P(3HB-co-3HV) may be a suitable packaging material for frozen or refrigerated products.

Keywords

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); stability; secondary crystallisation; thermal degradation; thermal properties; mechanical testing

1. Introduction

Poly(3-hydroxybutyrate) (P(3HB)) and its copolymers have received considerable attention in the packaging field as a result of their biodegradability,¹⁻³ and in some respects their similarity to commercial polymers such as polypropylene (PP) and polyethyleneterephthalate (PET).^{4,5} They are a class of polymers formed by various bacteria in environments where there is an excess carbon source and limited oxygen, nitrogen or phosphorous.^{6,7} In this bacterial synthesis, the polymer makes up around 90 % of the dry cell mass and has a number average molecular weight in the order of 10^5 to 10^6 Da. The stereospecifity of the bacterial production route generates stereochemically pure P(3HB) which leads to crystallinities of over 50 %.⁷ However, the high degree of crystallinity results in an elevated Young's modulus and low elongation to break; this has prevented the large scale commercial use of P(3HB).^{8,9} Numerous researchers have attempted to improve the properties of P(3HB) through copolymerising with hydroxyvalerate, via alternating the bacterial feed stock, to form P(3HB-co-3HV).^{10,11} This copolymerisation has been shown to successfully improve the mechanical properties of P(3HB), with an increasing HV content decreasing the crystallinity of the material.^{1,12-14} The processing of P(3HB) also has limitations as the polymer is very unstable in the melt resulting in bond cleavage and degradation of the material.^{15,16} This results in a very small processing window.

Another major issue surrounding the commercial use of P(3HB) and its copolymers is their ability to undergo secondary crystallisation over time which manifests as a progressive reduction in ductility.^{17,18} Secondary crystallisation occurs, following primary crystallisation, when the glass transition temperature (T_g) of a polymer remains below the storage temperature. The polymer chains in the amorphous regions possess a degree of mobility which allows them to continue the crystallisation process via thickening of the existing crystalline lamellae and infilling between the lamellae layers.¹⁹⁻²²

Several studies have shown that storage of P(3HB) and its copolymers at room temperature causes the crystallinity to increase and with that the physical properties of the material to change, 17,18,23 but studies involving storage at elevated temperatures have not been reported. Heating the material to high temperatures following long term storage at room temperature has been shown to improve the properties of P(3HB-co-3HV)¹⁷ or P(3HB),¹⁸ due to reorganisation of the lamellae, but reported annealing times were less than 12 hours.

Therefore, this study aims to address this limitation by assessing the temperature dependence of the long-term stability of P(3HB-co-3HV). In this study, the effects of long-term storage of P(3HB-co-3HV) at a wide range of temperatures, between the glass transition temperature and melting point (T_m), is reported. The effect of storage time and temperature on the crystallinity, thermal stability and mechanical properties is characterised using differential scanning calorimetry (DSC), dynamic mechanical thermal analysis (DMTA), and tensile testing. The results are considered in the context of secondary crystallisation and degradation.

2. Materials and Methods

2.1. Materials

Poly(3-hydroxybutyrate-co-3-valerate) (P(3HB-co-3HV)) (Tianan ENMAT Y1000P) containing 3 wt% valerate was supplied by Helian Polymers (Venlo, Netherlands) and used as received.

2.2. Methods

2.2.1. Sample Preparation

Plaques of P(3HB-co-3HV) were produced by compression moulding using a Moore E1127 hydraulic hot press (George E. Moore & Sons Ltd, Birmingham, UK) preheated to 190 °C. Briefly, the polymer pellets (8 g) were placed into a mould (152 x 158 x 0.266 mm) and inserted into the press. The mould was allowed to warm for 5 minutes before a load of 10 tonnes was applied for a further 3 minutes. The plaques were cooled in-situ by passing water through the platens before removal. This imposed a cooling rate of approximately 17 °C min⁻¹. Thicker plaques were also produced from 43 g of pellets in a 150 x 180 x 1 mm mould, using the same conditions.

Samples were cut from the plaques, as detailed in the appropriate experimental sections below, and stored at -22, 7, 20, 50, 75, 100, 125 and 150 °C. This range was selected as it covers temperatures between the T_g and T_m , and includes a temperature below the T_g to act as a control. Additional samples were also retained for day zero analysis. As the preparation of the samples and analyses were performed at room temperature, handling time of the samples was kept to a minimum to limit secondary crystallisation.

2.2.2. Differential Scanning Calorimetry (DSC)

Discs of 5 mm in diameter with a typical mass of 4.42 ± 2.37 mg were cut from the 0.266 mm plaques and stored at a range of temperatures. A Mettler Toledo DSC 1 (Mettler Toledo, Schwerzenbach, Switzerland), calibrated with indium and zinc standards, was used to determine the melting point and crystallinity of the P(3HB-co-3HV) samples during storage. All experiments were conducted under a nitrogen flow rate of 50 ml min⁻¹. A standard procedure was adopted in which the samples were weighed into 40 µl aluminium DSC pans

(Mettler Toledo), capped with aluminium DSC lids (Mettler Toledo) and sealed with a press (Mettler Toledo). Samples were heated from -40 to 220 °C at a constant rate of 50 °C min⁻¹ to limit melt recrystallization/reorganisation. The melting point was taken at the peak of the transition. The degree of crystallinity of P(3HB-co-3HV) was determined by drawing a linear baseline from the first onset of melting to the last trace of crystallinity and the enthalpy of fusion (ΔH_f) was then calculated from the area under the endotherm (Equation 1) using a ΔH^0_f value of 146 J g⁻¹ as reported for P(3HB).²⁴ To the authors knowledge no enthalpy of fusion has been published for the copolymer containing 3 wt% 3HV however, numerous researchers have used this P(3HB) value when analysing P(3HB-co-3HV) with low concentrations of HV.²⁵⁻²⁷

$$X_c = \frac{\Delta H_f}{\Delta H_f^0}$$
 Equation 1

2.2.3. Dynamic Mechanical Thermal Analysis (DMTA)

Rectangular samples (13 mm x 55 mm) were prepared from the 1 mm thick plaques. DMTA was carried out using a NETZSCH DMA 242 cell with a TASC 414/3 controller and a PC running NETZSCH DMA measurement channel 18 software. The loading geometry was 3-point bend and the free bending length was 40 mm for all samples. Liquid nitrogen was used to cool the sample to -40 °C prior to the start of the run and temperature scans from -40 to 70-95 °C were performed with a heating rate of 1 °C min⁻¹. The target deflection was set to 240 μ m for all samples apart from those stored at 150 °C where the deflection was 120 μ m. This was because the samples stored at this temperature were too brittle to enable measurement at deflections beyond 120 μ m. The storage modulus and loss tangent were recorded as function of temperature at three frequencies; 1, 10 and 33.33 Hz.

2.2.4. Mechanical Testing

Identical dog-bone shaped samples (gauge length 26 mm; width 4 mm; thickness $0.214 \pm 0.087 \mu$ m) were cut from the plaques using a Wallace cutter and stored at a range of temperatures. The Young's modulus, ultimate tensile strength and elongation to break of the samples were determined at ambient temperature using an Instron 5566 (High Wycombe, UK) mechanical tester equipped with a 10 kN load cell. At various time points, the samples were removed from their storage condition and allowed to warm/cool to room temperature for 10 minutes prior to analysis. The edges of the dog-bone were also smoothed with P800 emery paper. Samples were extended at a rate of 2 mm min⁻¹ until failure. Three samples were analysed for each time point and the average calculated.

2.2.5. Gel Permeation Chromatography (GPC)

GPC was used to examine the effect of storage at 150 °C on the molecular weight of P(3HBco-3HV). Samples (50 mg) were cut from the thinner plaques at specific time intervals and refluxed in chloroform (50 ml) at 85 °C until dissolved. The solutions were passed through a 0.22 μ m PVDF membrane prior to analysis. Samples were analysed by an Agilent Technologies (Berkshire, UK) LC 390-MDS instrument, comprising of two PLgel Mixed-D columns (5 μ m) at 30 °C, a PLgel guard column (5 μ m) and a differential refractive index (DRI) detector at the University of Warwick (UK). An injection volume of 100 μ l was used with a chloroform mobile phase at a flow rate of 1 ml min⁻¹. Polystyrene standards were used for calibration.

2.2.6. Mass Loss

The mass of samples (~30 mg) cut from the 1 mm thick plaques was analysed periodically over time using a Perkin Elmer Autobalance Ad-2.

3. Results and Discussion

3.1. Thermal analysis

P(3HB-co-3HV) is well-known to undergo secondary crystallisation over time at room temperature, causing embrittlement of the material and therefore a deterioration in the mechanical properties.^{17,18} Analysis of P(3HB-co-3HV) during storage at -22 and 7 °C showed no significant change in the crystallinity over 4 weeks (Figure 1).

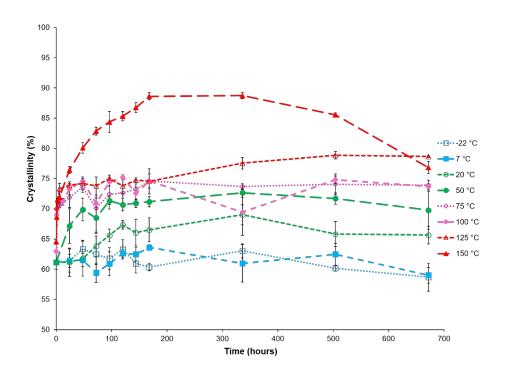


Figure 1: Change in crystallinity during storage at a range of temperatures. Error bars given as ± 1 standard deviation from the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

As the T_g of P(3HB-co-3HV) is approximately 4 °C,⁷ the amorphous phase within the samples is glassy at -22 °C and therefore largely immobilised such that increases in crystallinity via secondary crystallisation cannot occur. At temperatures above the Tg, the crystallinity was found to increase with time, with the majority of the change occurring in the first few days, which is in accordance with previous studies on P(3HB), in which the samples were stored at room temperature.¹⁷ Following this initial rapid increase in crystallinity the rate of change begins to reduce as the remaining amorphous material becomes more constrained, restricting chain mobility and with that the ease with which the amorphous phase can transform; an observation that has been reported previously using dielectric analysis.²¹ The exception to this is the samples stored at 150 °C where, although the crystallinity was found to initially rapidly increase, it began to decline following storage for longer than 14 days. P(3HB) and its copolymers are known to be thermally unstable at temperatures close to the melting point^{16,28-30} and there is evidence to suggest that the molecular weight of P(3HBco-3HV) may start to decrease on annealing at temperatures as low as 160 °C.³¹ Renstad et al. observed a 50 % reduction in the molecular weight of P(3HB-co-3HV) following extrusion at 162 °C with an estimated residence time of 12 mins.³¹ It is therefore reasonable to assume that at 150 °C both secondary crystallisation and chain scission occur in parallel over time. A small reduction in the molecular weight has been reported to increase crystallinity.^{16,32} This can be attributed to increased chain mobility and potential for close packing in the lamellae, which may account for the dramatic increase in crystallinity seen here compared to the other temperatures. P(3HB) is reported to reach crystallinities of 80%³³, and previous researchers have shown the degradation of P(3HB-co-3HV) via chemical hydrolysis to result in crystallinities of up to 75%³²; however, this is lower than the 89% observed here. As degradation occurs primarily in the amorphous regions, due to the structural rearrangement needed to form the intermediate cyclic ring, it is this material that will be lost via degradation first. Therefore, in addition to the increase in crystallinity created by secondary crystallisation and easier packing of the degraded shorter chains, the complete breakdown of sections of the amorphous region also occurs, leading to a higher than expected crystallinity. After one week, the degradation becomes more severe and not only proceeds in the amorphous phase, but also through the thickness of the lamella via the chain ends, as shown by the reduction in crystallinity.

In contrast to primary crystallisation, where Avrami analyses have shown the maximum rate of crystallisation to occur near to the midpoint between the T_g and T_m (80 °C in the case of P(3HB-co-3HV)³⁴ the rate of secondary crystallisation was found to increase with temperature, which can be explained by the non-nucleated growth of the existing lamella regions. In primary crystallisation there is a balance between low temperatures required for nuclei formation and high temperatures to provide sufficient chain mobility for spherulite growth. As secondary crystallisation occurs due to further chain rearrangement following primary crystallisation, leading to lamellae thickening and/or infilling, nucleation is not required. As a result, high temperatures are more favourable due to the increased chain mobility and free volume afforded to the polymer.

The melting points of the samples were also analysed periodically to assess any variation due to secondary crystallisation of the material (Figure 2). As expected, no change in the melting point was observed at the lower temperatures where the crystallinity remained consistent throughout storage. At temperatures in excess of 50 °C the melting point was found to increase over time in accordance with increased crystallinity (Figure 1). As the melting point is related to the thickness of the lamella and one method of secondary crystallisation is lamella thickening, it therefore follows that the melting point will increase on storage. At 150 °C the melting point was found to increase up to a time point of 100 hours, but then decrease to a melting temperature below that observed prior to storage. This can be ascribed to the

onset of degradation which has been reported previously.^{16,35} Although the temperatures adopted in these papers are greater than the 150 °C used here (190 and 180 °C respectively) it is probable that the far greater exposure times adopted in this study (up to 672 hours compared to 80 and 300 minutes) produced similar effects.

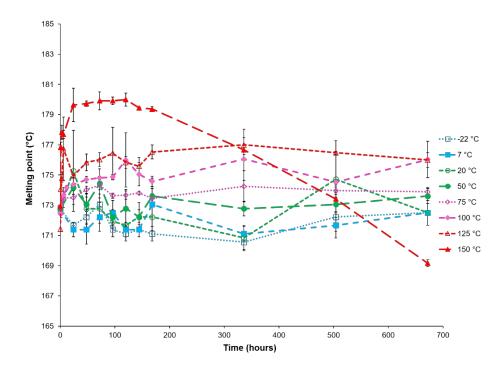


Figure 2: Effect of storage at a range of temperatures on the melting point of PHB-co-HV. Error bars are given as ±1 standard deviation from the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

The glass transition temperature could not be detected using DSC due to the low levels of amorphous material present. However, DMTA was able to resolve the α relaxation and the glass transition was then defined from the associated peak in tan δ . Analysis of the glass transition temperature over time at -22 and 7 °C showed negligible changes throughout the study due to the lack of chain mobility. Each of the traces displayed an asymmetric distribution with the high temperature side of the peak being broader than the left (Figure 4).

This is typical of a semi-crystalline polymer and can be ascribed to the constrainment of the mobile amorphous chains adjacent to the lamella surface (the region referred to as the rigid amorphous fraction, or RAF, in the three-phase model). Above 20 °C, with the exception of 150 °C, the T_g was found to rise over time with the greatest changes occurring at the higher storage temperatures (Figure 3).

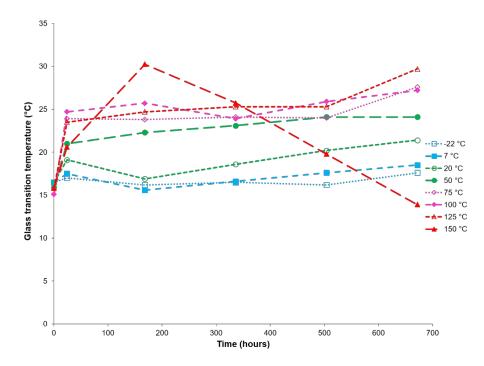


Figure 3: Effect of storage at a range of temperatures on the glass transition temperature of PHB-co-HV. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

In addition, the peak height of the α relaxation (which is related to the proportion of amorphous material³¹) was found to decrease with time which is consistent with the continued development of crystallinity (Figure 4a). These results are in accordance with the development of crystallinity with time shown above (Figure 1) in that the glass transition temperature reflects the mobility of the chains in the amorphous phase and the constraints imposed by the secondary crystallisation process. These results also concur with data

reported for poly(lactic acid).^{36,37} The variation of glass transition temperature with storage time at 150 °C is also in accordance with the variation of crystallinity under the same conditions, that is, an initial increase followed by a decline due to the onset of degradation. The behaviour of the α relaxation at 150 °C reflects the interplay between the secondary process and the degradation observed above (Figure 4b). Up to a storage time of 168 hours, the variation in peak profile is similar to that observed at the other storage temperatures i.e. there is a reduction in peak height and an increase in peak temperature, both driven by the secondary crystallisation process. However, on storage for time periods longer than 168 hours, the effects of degradation begin to dominate in that the peak temperature (Tg) begins to decrease. It is interesting to note the peak profile at storage times of 504 and 762 hours i.e. there is a symmetric broadening which can be ascribed to the effects of both secondary crystallisation and degradation. Secondary crystallisation further constrains the RAF which extends the α relaxation to elevated temperatures while degradation in (presumably) the mobile amorphous fraction results in chain scission and the formation of smaller, less constrained polymer chains.

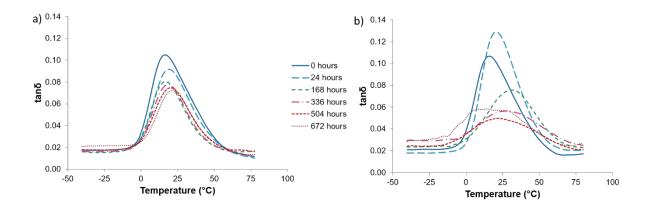


Figure 4: DMTA tano curves during storage at a) 20 °C and b) 150 °C

3.2. Mechanical Properties

Figure 5 shows the change in Young's modulus with time at the various storage temperatures. The results again show no change to the samples held at -22 °C due to the lack of polymer chain mobility below the glass transition temperature. With the exception of storage at 150 °C, the Young's modulus increased over time which is consistent with progressive constrainment of the mobile amorphous regions by the development of secondary crystallisation. This has been observed by other authors albeit following storage at room temperature.^{17,18}

Up to storage temperatures of 125 °C there was a significant change in modulus during the first day of storage, but this was followed by a more limited increase in modulus up to 180 hours (Figure 5). This observation is consistent with the increases observed in both the degree of crystallinity (Figure 1) and the glass transition temperature (Figure 3). At a storage temperature of 150 °C there was clear evidence of degradation as it was not possible to mount the samples for tensile testing; the samples either failed on handling or in the grips. The case of 125 °C showed evidence of both secondary crystallisation and degradation in that there was a more modest increase in modulus.

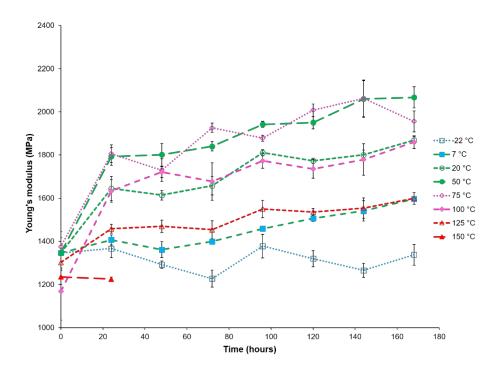


Figure 5: Change in the Young's modulus of PHB-co-HV during storage at a range of temperatures for 1 week. Error bars are given as ±1 standard deviation of the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

The ultimate tensile strength (UTS) was also found to vary with storage time with an increase observed throughout the study for the majority of the storage temperatures (Figure 6). As noted with each of the other properties, no significant change occurred in the samples stored at -22 and 7 °C. For storage temperatures between 20 and 75 °C the UTS increased in accordance with the increases in crystallinity observed previously, whereas, at 125 °C the UTS was observed to decrease following 5 days storage. At a storage temperature of 150 °C, the onset of the reduction in UTS appeared to be almost immediate; samples stored for a time period of longer than one day failed on handling or in the grips and it was impossible to determine the UTS. Previous work with P(3HB-co-3HV) has shown degradation to occur during annealing at temperatures of 180 °C and above for 5 minutes.²⁸ Furthermore, a reduction in molecular weight associated with degradation has been reported at temperatures as low as 160 °C following extrusion.³¹ The results presented in Figure 6 indicate that P(3HB-co-3HV) is thermally unstable well below the melting temperature and that degradation readily proceeds at temperatures at least as low as 125 °C. Up to a time point of five days, at 125 °C the effects of degradation are hidden to a degree by the increase in secondary crystallisation.

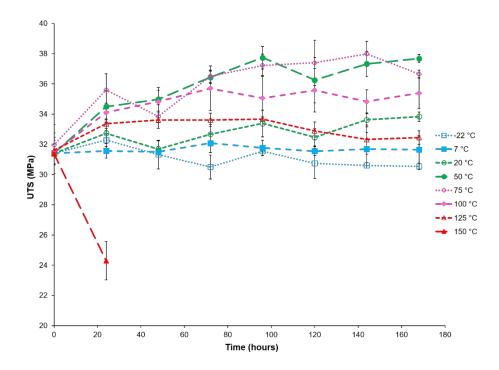


Figure 6: Effect of storage for 1 week at a range of temperatures on the UTS of PHB-co-HV. Errors bars are given as ± 1 standard deviation from the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

The elongation to break decreased with time on storage at all temperatures apart from -22 $^{\circ}$ C (Figure 7). This observation is again consistent with data reported by other authors that have conducted a time-resolved mechanical property study at ambient conditions.^{17,18}

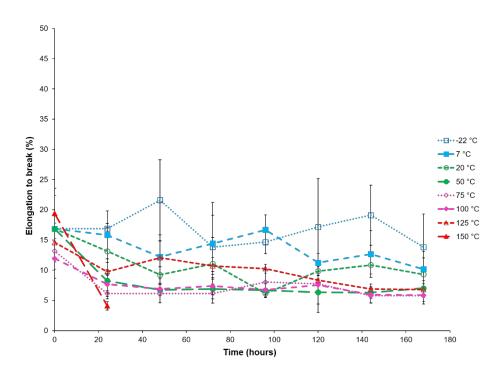


Figure 7: Change in the elongation to break of PHB-co-HV following storage at a range of temperatures for 1 week. Errors bars given as ±1 standard deviation from the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

3.3. Degradation at elevated temperatures

During the analyses, it was interesting to note that there was an obvious discolouration in the samples that had been stored at 150 °C, and to a lesser extent, those that had been stored at 125 °C (Figure 8). The observation strongly suggests that degradation is readily occurring at these temperatures. No obvious discoloration was observed in the samples stored at temperatures below 125 °C. Previous researchers have reported discolouration of P(3HB-co-3HV) following extrusion at 176-177 °C which they attributed to degradation and a reduction in the molecular weight.³¹

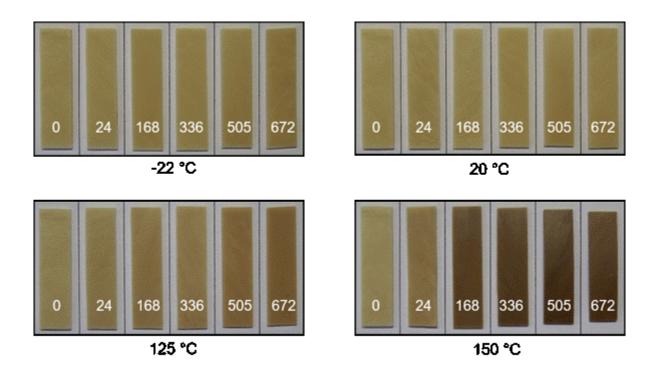


Figure 8: Discolouration of samples stored at elevated temperatures for the time duration labelled

The degradation pathway at elevated temperatures is widely reported to be random chain scission of the ester groups via β -elimination,^{16,29,30} with a 60% reduction in the molecular weight reported following annealing P(3HB) at 180 °C for 10 minutes.¹⁶ As this chain scission results in the formation of smaller molecular weight chains the occurrence of degradation during this study can be assessed by GPC. The molecular weight of P(3HB-co-3HV) was found to decrease markedly over time indicating chain cleavage and degradation (Figure 9).

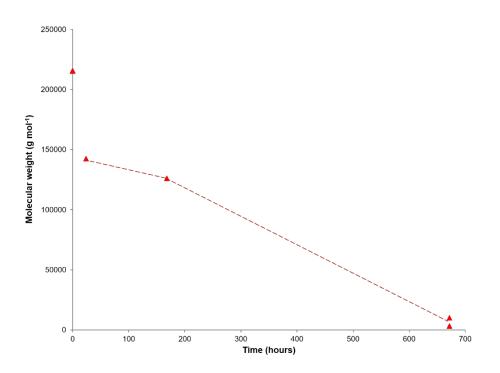


Figure 9: GPC analysis of the reduction in the molecular weight of PHB-co-HV during storage at 150 °C. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

Interestingly two overlapping peaks were observed in the chromatogram, corresponding to molecular weights of 3373 and 10100 g mol⁻¹, after 4 weeks exposure to 150 °C. This observation supports the idea that shorter chains are formed at this temperature which are able to crystallise relatively easily such that the overall crystallinity increases rapidly, that is, the development of crystallinity is facilitated by the degradation process.

Sample mass was also found to vary with time at elevated storage temperatures (Figure 10). In all cases above 50 °C there was a slight initial mass loss over the first 24 hours (probably due to the loss of absorbed moisture). Following this, the mass of the samples stored at 100 °C and below remained constant for the duration of the study. However, on storage at 125 °C an initial mass loss of 0.26 % was observed due to evaporation of surface water (Figure 10) followed by an additional 0.36 % over the course of the study, indicative of degradation of

the material. A more dramatic reduction in the mass was observed at 150 °C, in which an initial loss of 0.43 % was followed by a 27.86 % reduction over 4 weeks indicating severe degradation (Figure 10a). A similar percentage weight loss was also observed when unprocessed P(3HB-co-3HV) pellets were subjected to the same conditions (data not shown).

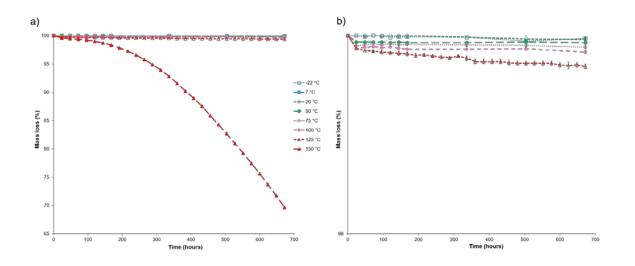


Figure 10: Mass loss of PHB-co-HV stored at a range of temperatures. Error bars are equivalent to \pm 1 standard deviation from the mean. Dashed lines have been superimposed between time points as a visual aid to guide the eye through the data sets.

Previous research has shown that at temperatures greater than the melting point further degradation of P(3HB) and P(3HB-co-3HV) can occur, leading to the formation of crotonic acid and 2-pentenoic acid as the HB and HV units break down respectively.^{30,38,39} The boiling point of 2-pentenoic acid is 106 °C and will therefore evaporate immediately if produced during degradation. In addition, although the boiling point of crotonic acid (180 °C) is lower than the storage temperature, it is probable that due to the prolonged storage time these monomers will also evaporate therefore, the mass loss shown in Figure 10 is likely to originate from the loss of both degradants.

4. Conclusions

This study aimed to address limitations in previous work in the area of P(3HB-co-3HV) secondary crystallisation. This was achieved by systematically extending both the range of storage temperatures and the timescale. At storage temperatures below 125 °C, P(3HB-co-3HV) appears not to degrade and the increases in degree of crystallinity, melting point, glass to liquid transition temperature, modulus and strength can be attributed to the secondary crystallisation process. In this temperature range the development of secondary crystallinity is facilitated by increasing temperature. In contrast, at 150 °C there is a deterioration in all the properties noted above and given the reduction in molecular weight (and discolouration), this can be attributed to degradation. The peak profile for the α relaxation at 150 °C reveals significant symmetric broadening due to the effects of the secondary crystallisation process and degradation. High temperature broadening caused by further constrainment of the RAF and an increase in the mobility in the mobile amorphous fraction (MAF) as a result of the formation of smaller, less constrains chains. At 125 °C, it is apparent that the processes of secondary crystallisation and degradation occur in parallel and at this temperature there is also an interplay between these processes in that the effects of degradation are masked to a degree by the continuation of secondary crystallisation. The significant observation to emerge from the results is that the degradation process remains active well below the melting point, and that the long-term stability of P(3HB-co-3HV) is not only affected by secondary crystallisation, but also sub-melting point degradation. In addition, as no significant changes to the thermal or mechanical properties were observed during storage at -22 C and 7 °C it suggests that P(3HB-co-3HV) may be a suitable packaging material for frozen or refrigerated products.

5. Acknowledgements

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6. References

- Luzier, W. D. Materials derived from biomass/biodegradable materials. *Proceedings of the National Academy of Sciences* **89**, 839-842, doi:10.1073/pnas.89.3.839 (1992).
- 2 Mergaert, J., Webb, A., Anderson, C., Wouters, A. & Swings, J. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. *Applied and Environmental Microbiology* **59**, 3233-3238, doi:0099-2240/93/103233-06\$02.00/0 (1993).
- 3 Tansengco, M. & Dogma, I. Microbial degradation of poly-β-hydroxybutyrate using landfill soils. *Acta Biotechnologica* **19**, 191-203, doi:10.1002/abio.370190302 (1999).
- 4 Bugnicourt, E., Cinelli, P., Lazzeri, A. & Alvarez, V. Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *Express Polym. Lett.* **8**, 791-808, doi:10.3144/expresspolymlett.2014.82 (2014).
- 5 Miguel, O. & Iruin, J. J. Evaluation of the transport properties of poly(3-hydroxybutyrate) and its 3-hydroxyvalerate copolymers for packaging applications. *Macromol. Symp.* **144**, 427-438, doi:10.1002/masy.19991440140 (1999).
- 6 Lee, S. Y. Bacterial polyhydroxyalkanoates. *Biotechnol. Bioeng.* **49**, 1-14, doi:0006-3592/96/010001-14 (1996).
- 7 Doi, Y. *Microbial Polyesters*. (VCH Publishers, 1990).
- 8 Bibers, I., Tupureina, V., Dzene, A. & Kalnins, M. Improvement of the deformative characteristics of poly-beta-hydroxybutyrate by plasticization. *Mech. Compos. Mater.* **35**, 357-364, doi:10.1007/bf02259726 (1999).
- 9 Asrar, J. & Gruys, K. in *Biopolymers* Vol. 4 *Polyesters III. Application and commercial products* (eds Y. Doi & A. Steinbuchel) (Wiley-VCH 2004).
- 10 You, J. W., Chiu, H. J., Shu, W. J. & Don, T. M. Influence of hydroxyvalerate content on the crystallization kinetics of poly(hydroxybutyrate-co-hydroxyvalerate). *J. Polym. Res.-Taiwan* **10**, 47-54, doi:10.1023/a:1023958014221 (2003).
- 11 Holmes, P. A. Applications of PHB A microbially produced biodegradable thermoplastic *Physics in Technology* **16**, 32-36, doi:10.1088/0305-4624/16/1/305 (1985).
- 12 Owen, A. J., Heinzel, J., Skrbic, Z. & Divjakovic, V. Crystallization and melting behavior of PHB and PHB/HV copolymer *Polymer* **33**, 1563-1567, doi:10.1016/0032-3861(92)90139-n (1992).
- 13 Modi, S., Koelling, K. & Vodovotz, Y. Assessment of PHB with varying hydroxyvalerate content for potential packaging applications. *Eur. Polym. J.* **47**, 179-186, doi:10.1016/j.eurpolymj.2010.11.010 (2011).
- 14 Barham, P. J., Barker, P. & Organ, S. J. Physical-properties of poly(hydroxybutyrate) and copolymers hydroxybutyrate and hydroxyvalerate *FEMS Microbiol. Lett.* **103**, 289-298, doi:10.1016/0378-1097(92)90322-f (1992).

- 15 Vogel, C. *et al.* Thermal degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrateco-3-hydroxyhexanoate) in nitrogen and oxygen studied by thermogravimetric-Fourier transform infrared spectroscopy. *Applied Spectroscopy* **61**, 755-764, doi:10.1366/000370207781393370 (2007).
- 16 Janigova, I., Lacik, I. & Chodak, I. Thermal degradation of plasticized poly(3-hydroxybutyrate) investigated by DSC. *Polym. Degrad. Stabil.* **77**, 35-41, doi:10.1016/s0141-3910(02)00077-0 (2002).
- 17 Biddlestone, F., Harris, A., Hay, J. N. & Hammond, T. The physical ageing of amorphous poly(hydroxybutyrate). *Polym. Int.* **39**, 221-229, doi:0959-8103/96/\$09.00 (1996).
- 18 Dekoning, G. J. M. & Lemstra, P. J. Crystallisation phenoma in bacterial poly(R)-3hydroxybutyrate. 2. Embrittlement and rejuvenation *Polymer* 34, 4089-4094, doi:10.1016/0032-3861(93)90671-v (1993).
- 19 Kolb, R., Wutz, C., Stribeck, N., von Krosigk, G. & Riekel, C. Investigation of secondary crystallization of polymers by means of microbeam X-ray scattering. *Polymer* **42**, 5257-5266, doi:10.1016/s0032-3861(00)00920-4 (2001).
- 20 Marand, H. & Huang, Z. Y. Isothermal lamellar thickening in linear polyethylene: Correlation between the evolution of the degree of crystallinity and the melting temperature. *Macromolecules* **37**, 6492-6497, doi:10.1021/ma0497198 (2004).
- 21 Sics, I. *et al.* On the relationship between crystalline structure and amorphous phase dynamics during isothermal crystallization of bacterial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymers. *Biomacromolecules* **2**, 581-587, doi:10.1021/bm0155266 (2001).
- 22 Wang, Z. G., Hsiao, B. S., Sauer, B. B. & Kampert, W. G. The nature of secondary crystallization in poly(ethylene terephthalate). *Polymer* **40**, 4615-4627, doi:10.1016/s0032-3861(99)00067-1 (1999).
- Dekoning, G. J. M., Scheeren, A. H. C., Lemstra, P. J., Peeters, M. & Reynaers, H.
 Crystallisation phenomena in bacterial poly (R)-3-hydroxybutyrate .3. Toughening via texture changes *Polymer* 35, 4598-4605, doi:10.1016/0032-3861(94)90809-5 (1994).
- 24 Barham, P. J., Keller, A., Otun, E. L. & Holmes, P. A. Crystallization and morphology of a bacterial thermoplastic Poly-3-hydroxybutyrate *J. Mater. Sci.* **19**, 2781-2794, doi:10.1007/bf01026954 (1984).
- 25 Avella, M. *et al.* Novel synthesis blends between bacterial polyesters and acrylic rubber: A study on enzymatic biodegradation. *J. Environ. Polym. Degrad.* **3**, 49-60, doi:10.1007/bf02067793 (1995).
- 26 Thellen, C. *et al.* A Processing, Characterization and Marine Biodegradation Study of Melt-Extruded Polyhydroxyalkanoate (PHA) Films. *J. Polym. Environ.* **16**, 1-11, doi:10.1007/s10924-008-0079-6 (2008).
- 27 Rosa, D. D., Calil, M. R., Guedes, C. D. F. & Rodrigues, T. C. Biodegradability of thermally aged PHB, PHB-V, and PCL in soil compostage. *J. Polym. Environ.* **12**, 239-245 (2004).
- Kunioka, M. & Doi, Y. Thermal degradation of microbial copolymesters Poly(3hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate).
 Macromolecules 23, 1933-1936, doi:10.1021/ma00209a009 (1990).
- Nguyen, S., Yu, G. E. & Marchessault, R. H. Thermal degradation of poly(3hydroxyalkanoates): Preparation of well-defined oligomers. *Biomacromolecules* 3, 219-224, doi:10.1021/bm0156274 (2002).
- 30 Grassie, N., Murray, E. J. & Holmes, P. A. The thermal degradation of poly(-(D)-betahydroxybutyric acid) .2. Changes in molecular weight *Polym. Degrad. Stabil.* **6**, 95-103, doi:10.1016/0141-3910(84)90075-2 (1984).
- 31 Renstad, R., Karlsson, S. & Albertsson, A. C. Influence of processing parameters on the molecular weight and mechanical properties of poly(3-hydroxybutyrate-co-3-

hydroxyvalerate). *Polym. Degrad. Stabil.* **57**, 331-338, doi:10.1016/s0141-3910(97)00028-1 (1997).

- 32 Renstad, R., Karlsson, S. & Albertsson, A. C. The influence of processing induced differences in molecular structure on the biological and non-biological degradation of poly(3hydroxybutyrate-co-3-hydroxyvalerate), P(3-HB-co-3-HV). *Polym. Degrad. Stabil.* **63**, 201-211, doi:10.1016/s0141-3910(98)00092-5 (1999).
- 33 Holmes, P. A. in *Developments in crystalline polymers -2* (ed D.C. Bassett) (Elsevier Applied Science Publishers, 1988).
- 34 Peng, S. W. *et al.* Isothermal crystallization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Eur. Polym. J.* **39**, 1475-1480, doi:10.1016/s0014-3057(03)00014-4 (2003).
- 35 Zagar, E. & Krzan, A. SEC-MALS characterization of microbial polyhydroxyalkanoates. *Biomacromolecules* **5**, 628-636, doi:10.1021/bm030073l (2004).
- Lizundia, E., Petisco, S. & Sarasua, J. R. Phase-structure and mechanical properties of isothermally melt-and cold-crystallized poly (L-lactide). *J. Mech. Behav. Biomed. Mater.* 17, 242-251, doi:10.1016/j.jmbbm.2012.09.006 (2013).
- 37 Zuza, E. *et al.* Glass transition behavior and dynamic fragility in polylactides containing mobile and rigid amorphous fractions. *Polymer* **49**, 4427-4432, doi:10.1016/j.polymer.2008.08.012 (2008).
- Li, S.-D., Yu, P. H. & Cheung, M. K. Thermogravimetric analysis of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Journal of Applied Polymer Science* 80, 2237-2244, doi:10.1002/app.1327 (2001).
- 39 Yang, H. L. & Liu, J. J. Thermal analysis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) irradiated under vacuum. *Polym. Int.* **53**, 1677-1681, doi:10.1002/pi.1521 (2004).