

Effect of 3-hydroxyvalerate content on Thermal, Mechanical, and Rheological properties of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) biopolymers produced from fermented dairy manure

Maryam Abbasi ¹, Dikshya Pokhrel ¹, Erik R. Coats ², Nicholas M. Guho ² and Armando G. McDonald ^{1,*}

¹ Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, ID, USA

² Department of Civil and Environmental Engineering, University of Idaho, Moscow, ID, USA

* Correspondence: armandm@uidaho.edu; Tel.: +1-2088859454

Supplemental Material

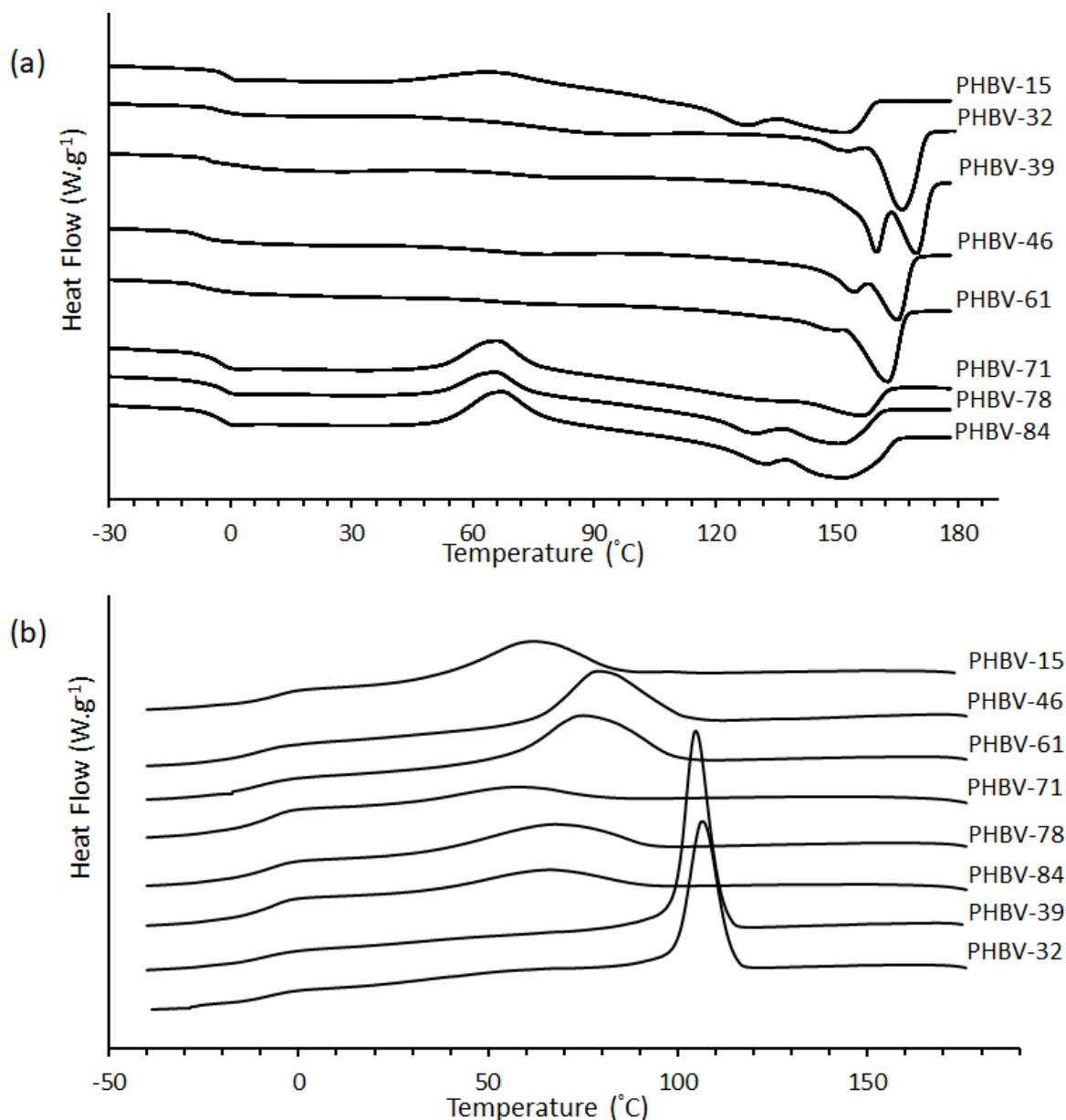


Figure S1. DSC thermograms for pure PHBV-15 to PHBV-84, (a) second heating scan; and (b) first cooling scan.

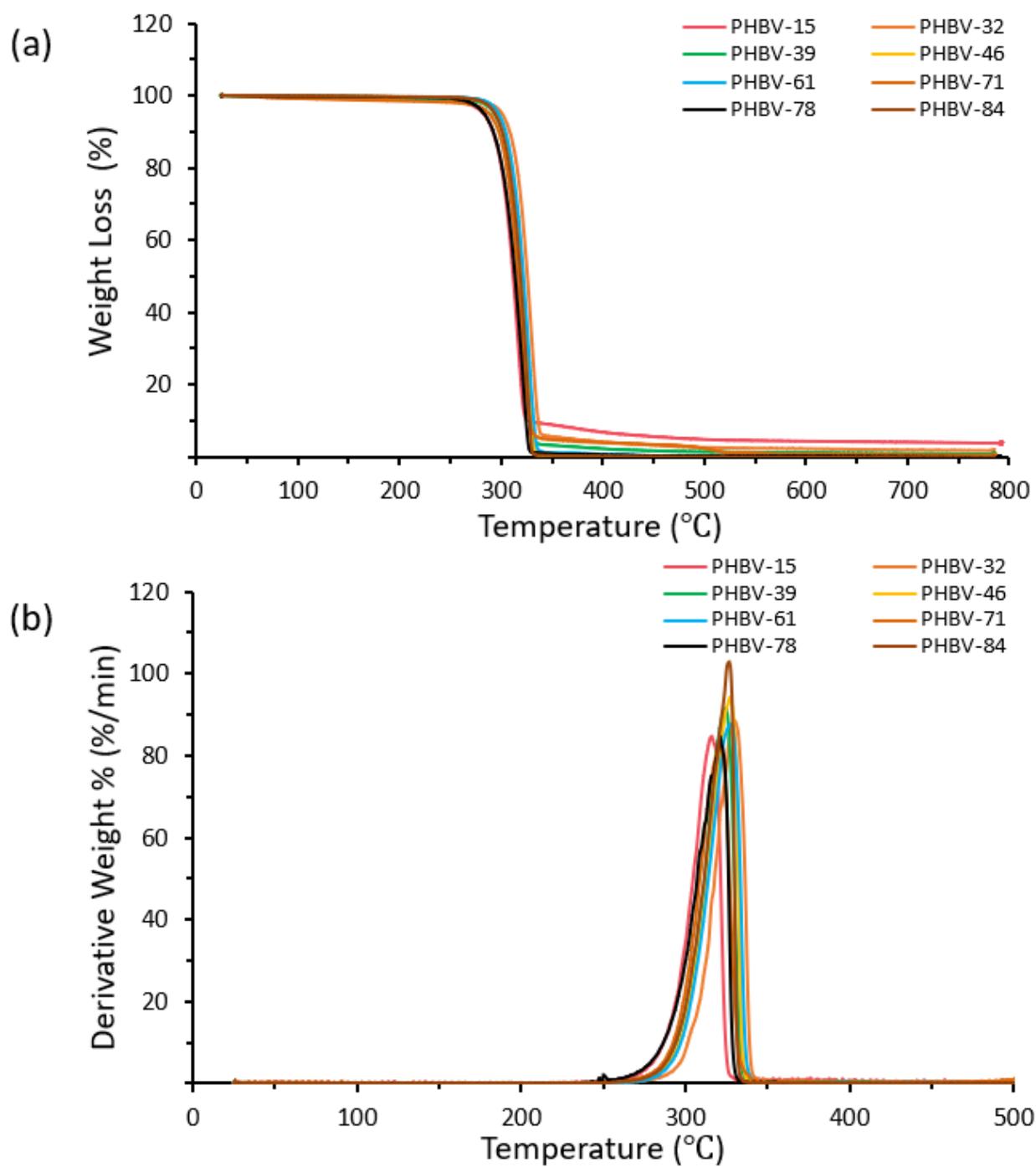


Figure S2. (a) TGA thermograms; and (b) DTG thermograms for pure PHBV-15 to PHBV-84.

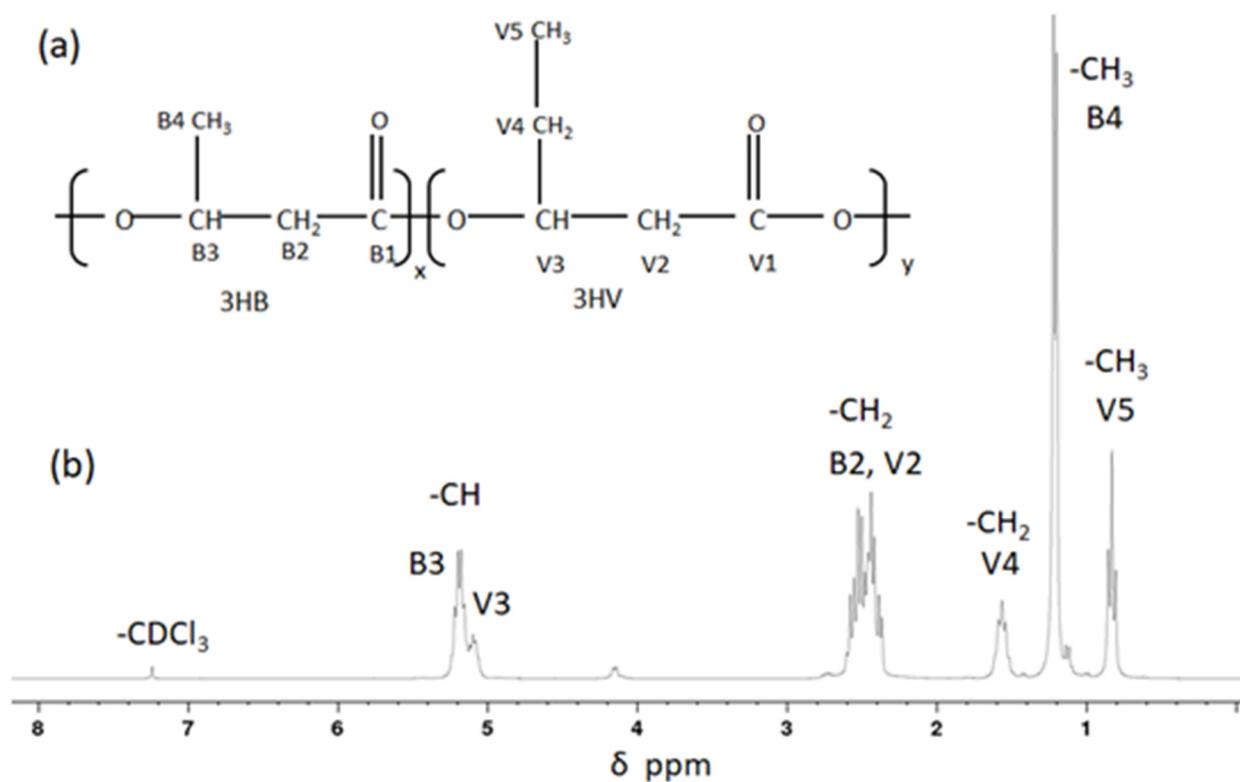


Figure S3. ^1H -NMR spectrum of PHBV-61, (a) structure; and (b) peak assignments.

Table S1. ^{13}C -NMR chemical shifts for carbonyl and methylene carbons in PHBV operational day 61.

Functional group	Chemical Shift (δ , ppm)	Sequence
C=O (B1, V1)	169.56	V*V
	169.38	B*V
	169.35	V*B
	169.18	B*B
CH ₂ (V2)	38.86	BV*B
	38.83	VV*B
	38.73	BV*V
	38.71	VV*V
CH ₂ (B2)	40.87	B*B
	40.83	B*V
CH ₂ (V4)	26.91	BV*B
	26.88	VV*B
	26.85	BV*V
	26.82	VV*V

The coefficient of R is defined as [1]:

$$R = \frac{L_V^R}{L_V^E} = \frac{L_B^R}{L_B^E} \quad (\text{Eq S1})$$

L_V^E and L_B^E indicate the number average lengths of HV and HB blocks in the copolymers on the experimental triad level which can be calculated by the following equations:

$$L_V^E = \frac{(F_{VVV} + F_{VVB} + F_{BVV} + F_{BVB})}{(F_{BVB} + F_{VVB})} \quad (\text{Eq S2})$$

$$L_B^E = \frac{(F_{BBB} + F_{VBB} + F_{BBV} + F_{VBV})}{(F_{VBV} + F_{VBB})}$$

F_{XYZ} shows the relative molar fraction of XYZ triad sequence. L_V^R and L_B^R are the number average lengths of HV and HB blocks gained from HB and HV units which are statistically random distributed in the copolymer and are defined by following equations:

$$L_B^R = K + 1 \quad (\text{Eq S3})$$

$$L_V^R = \frac{(K + 1)}{K}$$

$$K = \frac{[HB]}{[HV]}$$

$$[HB] = \frac{K}{(K + 1)}$$

$$[HV] = \frac{1}{(K + 1)}$$

K is the ratio between the concentration of HB and HV units in the copolymer [1].

The diad and triad sequence distribution of PHBV biopolymer samples were calculated using three models as following [2,3]:

(i) Bernoullian statistics model, the simplest random copolymer model, which describes a statistically random copolymer. Calculation was expressed with the experimental mole fraction of 3HV unit (F_V^E).

$$F_{VV} = (F_V^E)^2 \quad (\text{Eq S4})$$

$$F_{VB} = F_{BV} = F_V^E (1 - F_V^E)$$

$$F_{BB} = (1 - F_V^E)^2$$

$$F_{VVV} = (F_V^E)^3$$

$$F_{BVV} = F_{VVB} = (F_V^E)^2 (1 - F_V^E)$$

$$F_{BVB} = F_V^E (1 - F_V^E)^2$$

Where F_{VV} , F_{VB} , F_{BV} , and F_{BB} (F_{XY} represents the mole fraction of XY sequence). The parameters associated with the superscript E shows the values that can be determined experimentally.

(ii) First-order Markovian model that is applicable on block, random and alternative copolymers. The relations can be described as follows:

$$P_{VV} = \frac{F_{VV}^E}{F_V^E} \quad (\text{Eq S5})$$

$$P_{VB} = \frac{F_{VB}^E}{F_V^E}$$

$$P_{BV} = \frac{F_{BV}^E}{F_B^E}$$

$$P_{BB} = \frac{F_{BB}^E}{F_B^E}$$

$$F_{VV} = \frac{P_{VV} P_{BV}}{(P_{VB} + P_{BV})}$$

$$F_{VB} = F_{BV} = \frac{P_{VB} P_{BV}}{(P_{VB} + P_{BV})}$$

$$F_{BB} = \frac{P_{VB} P_{BB}}{(P_{VB} + P_{BV})}$$

$$F_{VVV} = \frac{P_{VV}^2 P_{BV}}{(P_{VB} + P_{BV})}$$

$$F_{BVV} = F_{VVB} = \frac{P_{VV} P_{BV} P_{VB}}{(P_{VB} + P_{BV})}$$

$$F_{BVB} = \frac{P_{VB}^2 P_{BV}}{(P_{VB} + P_{BV})}$$

Where P_{ij} ($i=j= B$ or V) is the conditional possibility of j addition to the i last unit at the propagating chain end i with the relations that $P_{BV}+P_{BB}=1$ and $P_{VB}+ P_{VV}=1$

(iii) A mixture of two Bernoullian random copolymers. If two Bernoullian model copolymers with the 3HV mole fractions of A and B are mixed with a molar ratio of $X:(1-X)$, then the three values of A , B , and X can be determined from the molar fractions of 3HV-centered triad sequences through the following equations using the Newton Method and MATLAB R2014a software. A , B , X in these equations must be between 0 and 1.

$$F_{BVV}^E = F_{VVB}^E = A^2(1-A)X + B^2(1-B)(1-X) \quad (\text{Eq S6})$$

$$F_{BVB}^E = A(1-A)^2X + B(1-B)^2(1-X)$$

$$F_{VVV}^E = A^3X + B^3(1-X)$$

$$F_V = AX + B(1-X)$$

$$F_B = (1-A)X + (1-B)(1-X)$$

$$F_{VV} = A^2X + B^2(1-X)$$

$$F_{VB} = F_{BV} = A(1-A)X + B(1-B)(1-X)$$

$$F_{BB} = (1-A)^2X + (1-B)^2(1-X)$$

$$F_{VVV} = A^3X + B^3(1-X)$$

$$F_{BVV} = F_{VVB} = A^2(1-A)X + B^2(1-B)(1-X)$$

$$F_{BVB} = A(1-A)^2X + B(1-B)^2(1-X)$$

L_V^E is known as experimental number average sequence lengths of HV units, L_V^R is number average sequence length of randomly distributed HV units in copolymer, k is the ratio between the concentration of HV and HB units, P_{ij} 's are four conditional probabilities, and $r_1 r_2$ is the reaction index for PHBV.

References

1. Žagar, E.; Kržan, A.; Adamus, G.; Kowalczyk, M. Sequence distribution in microbial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) co-polyesters determined by NMR and MS. *Biomacromolecules* **2006**, *7*, 2210–2216.
2. Kamiya, N.; Yamamoto, Y.; Inoue, Y.; Chujo, R.; Doi, Y. Microstructure of Bacterially Synthesized Poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Macromolecules* **1989**, *22*, 1676–1682.
3. Wei, L.; Guho, N.M.; Coats, E.R.; McDonald, A.G. Characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) biosynthesized by mixed microbial consortia fed fermented dairy manure. *J. Appl. Polym. Sci.* **2014**, *131*.