CHARACTERIZATION OF THE NON-VOLATILE FRACTION FOCUSING ON THE IDENTIFICATION OF OLIGOMERS IN A PHA BIOPOLYMER PACKAGING USING LC-MS/MS

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INTRODUCTION

Polyhydroxyalkanoates (PHA) are biological and biodegradable polyesters produced naturally through bacterial fermentation of lipids and sugars. Nowadays, they have attracted increasing interest as a sustainable alternative to conventional plastics for food packaging. However, there is limited information on their chemical composition.

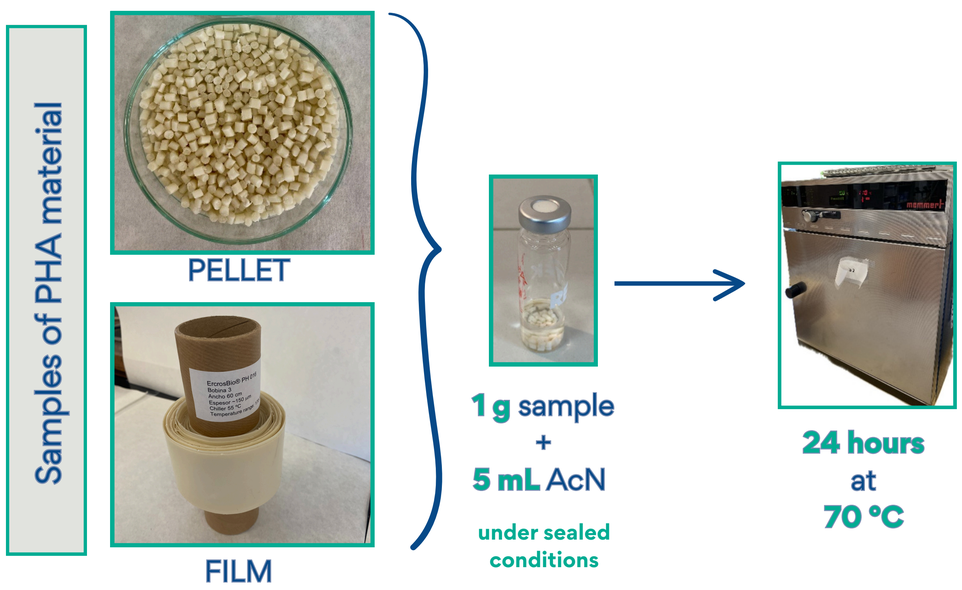
OBJECTIVES

The aim of the present research work was :

- To characterise the non-volatile fraction of a PHA based material extracts using LC-MS.
- To estimate the toxicity of the tentatively identified oligomers by an “in-silico” method.

METHODOLOGY

Extraction procedure



Non-volatile compound analysis

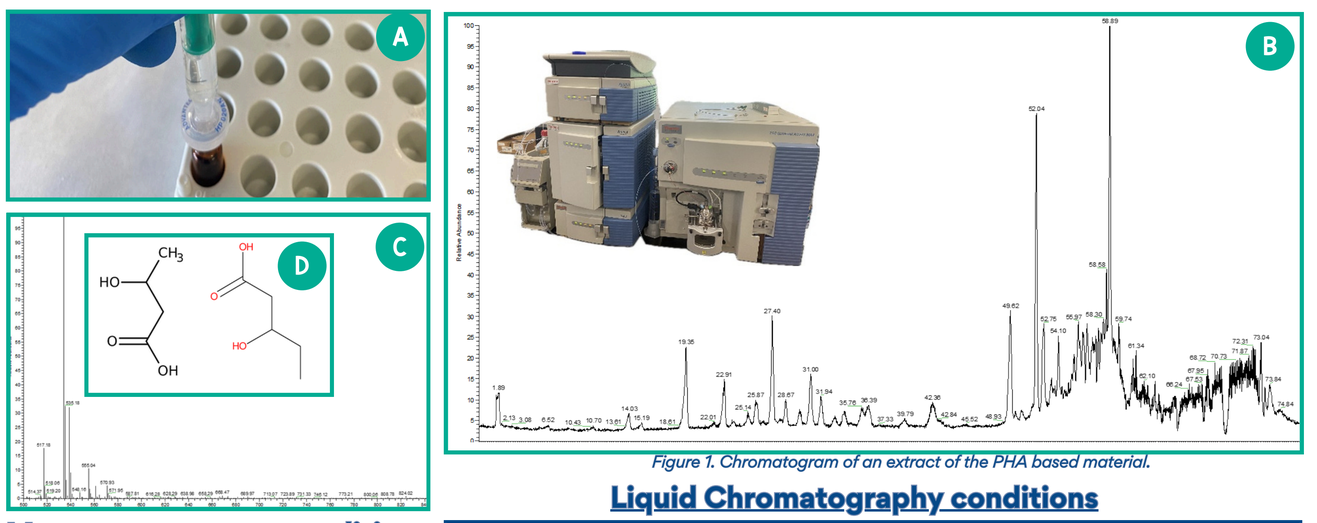


Figure 1. Chromatogram of an extract of the PHA based material.

Mass spectrometry conditions

- (ESI) Mode: Positive Electrospray ionization
- Full scan (m/z 100-1000)
- Spray Voltage: 3000 V
- Vaporizer Tª: 340 °C
- Capillary Tª: 350 °C
- Seath Gas: Nitrogen
- Seath Gas Pressure: 35 psi

Liquid Chromatography conditions

Mobile phase:
A: H_2O + 0.1% formic acid (v/v)
B: AcN + 0.1% formic acid (v/v)
Elution Gradient:

Time (min)	0	25	45	60	70	72	77
A (%)	80	50	0	0	0	80	80
B (%)	20	50	100	100	100	20	20

Column: Gemini C18 (150 mm x 3 mm, 5µm)
Flow rate: 0.40 mL/min
Injection volume: 10 µL
Column compartment Tª: 35 °C

- [A] Filtered extract in test vials.
- [B] LC Separation of PHA oligomers.
- [C] MS/MS Information (Mass Measurement MS).
- [D] Tentative Chemical Identification (Suspicious through prior knowledge of the material).

Toxicity estimation by ToxTree Software (based on Cramer’s rules to classify into low, moderate and high toxicity (classes I, II and III respectively).

RESULTS

- The study was focused on low molecular weight oligomers (<1000Da), since it assumed that higher molecular compounds are not absorbed through the gastrointestinal tract.
- Oligomers of 3-hydroxybutyrate (3HB) ranging from the trimer (n=3) up to undecamer (n=11), and oligomers of 3-hydroxyvalerate (3HV) ranging from the trimer (n=3) up to nonamer (n=9) were identified [Table 1].
- Both series oligomers were tentatively identified in pellets and films.
- *H, Na, K, NH_4* and *AcN* main adducts were detected in both formats, indicating interactions of the oligomers with various agents (Choi *et al.*, 2020; Hoque *et al.*, 2021).
- The theoretical toxicity of 3HB oligomers were Cramer I, while 3HV oligomers and 3HB oligomers (n=6 up to n=11) were Cramer III due to their greater structural complexity (Cramer *et al.*, 1978).

Table 1 *Table of identified oligomers. Oligomers detected in PHA samples (pellet and film).*

Tentative Oligomer type	*Formula*	*Molecular Ions Observed (M/z)*	*Aducts*	*Toxicity (Cramer's rules)*	*Pellet Format*	*Film Format*
3 HB Trimer	$C_{12}H_{20}O_7$	298.9, 315.0	*[M+Na]+, [M+K]+*	I	☑	☑
3HB Tetramer	$C_{16}H_{26}O_9$	363.1, 380.1, 385.0, 401.0	*[M+H]+, [M+NH_4]+, [M+Na]+, [M+K]+*	I	☑	☑
3HB Pentamer	$C_{20}H_{32}O_{11}$	449.1, 466.1, 471.1, 487.1	*[M+H]+, [M+NH_4]+, [M+Na]+, [M+K]+*	I	☑	☑
3 HB Hexamer	$C_{24}H_{38}O_{13}$	535.1, 552.1, 557.1, 573.0	*[M+H]+, [M+NH_4]+, [M+Na]+, [M+K]+*	III	☑	☑
3 HB Heptamer	$C_{28}H_{44}O_{15}$	638.8	*[M+NH_4]+*	III	☑	☑
3 HB Octamer	$C_{32}H_{50}O_{17}$	724.1, 729.0, 745.0	*[M+NH_4]+, [M+Na]+, [M+K]+*	III	☑	☑
3 HB Nonamer	$C_{36}H_{56}O_{19}$	810.1, 831.3	*[M+NH_4]+, [M+K]+*	III	☑	☑
3 HB Decamer	$C_{40}H_{62}O_{21}$	896.2, 901.05	*[M+NH_4]+, [M+Na]+*	III	☑	☑
3 HB Undecamer	$C_{44}H_{68}O_{23}$	982.2, 987.1	*[M+NH_4]+, [M+Na]+*	III	☑	☑
3 HV Trimer	$C_{15}H_{26}O_7$	319.1	*[M+H]+*	III	☑	☐
3 HV Tetramer	$C_{20}H_{34}O_9$	436.2, 457.1	*[M+NH_4]+, [M+K]+*	III	☑	☑
3 HV Pentamer	$C_{25}H_{42}O_{11}$	557.2	*[M+K]+*	III	☐	☑
3 HV Hexamer	$C_{30}H_{50}O_{13}$	657.5, 660.3	*[M+K]+, [M+AcN]+*	III	☑	☑
3 HV Heptamer	$C_{35}H_{58}O_{15}$	741.1, 757.2	*[M+Na]+, [M+K]+*	III	☐	☑
3 HV Octamer	$C_{40}H_{66}O_{17}$	860.3	*[M+AcN]+*	III	☑	☑
3 HV Nonamer	$C_{45}H_{74}O_{19}$	936.4	*[M+NH_4]+*	III	☑	☑

CONCLUSIONS

- The PHA based material analyzed contains oligomers from 3 up to 11 units and from 3 up to 9 units of 3HB and 3HV, respectively.
- Adducts such as H, Na, K, NH_4 and AcN were detected, suggesting interaction with agents during the analysis of the PHA.
- Toxicity estimation shows 3HB oligomers (n=3 up to n=5) have low toxicity (Cramer I), 3HV oligomers and 3HB oligomers (n=6 up to n=11) exhibit high toxicity (Cramer III).
- Future work will be focused on migration testing.

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