Tentative identification of non-volatile compounds in a biodegradable bio-based packaging material using a non-targeted method

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INTRODUCTION

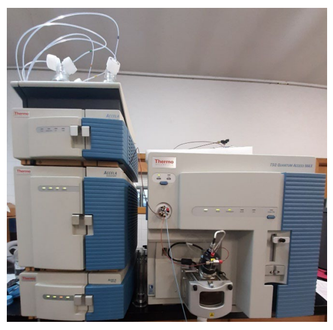
Bio-based polymers derived from renewable biological resources, and biodegradable polymers that will easily disintegrate and biodegrade in the environment, are being developed and promoted as an alternative to conventional petroleum-based non-biodegradable plastics to be utilized in food packaging. However, these materials generally do not perform as well as conventional plastics and require additional chemicals such as plasticizers, antioxidants, light and UV stabilizers, release agents, crosslinking agents, etc. Alternatively, the polymers are blended together or copolymerized to obtain materials with improved properties. Therefore, these polymers, like other food contact materials, can release low molecular weight components to food and may pose a health risk to consumers. The chemical safety of these sustainable materials has been scarcely studied.

The objective of the present work is to carry out a non-targeted analysis using liquid chromatography with mass spectrometry (LC-MS) to evaluate the presence of monomers and tentatively identify possible oligomers below 1000 Da extracted from polyhydroxybutyrate pellets (PHB), the most common polyhydroxyalkanoate. Also, to provide knowledge on which substances could be targeted in specific migration tests into food simulants or foods.

MATERIALS AND METHODS

Industry-provided PHB pellet

Extraction with acetonitrile (ACN) 40 °C/24 h



LC-MS

Column	Gemini C18 110 Å (150 mm × 3 mm, 5 µm)
Mobile phase	ACN and H_2O with 0.1% formic acid
Flow rate	0.4 mL/min
Injection volume	10 µL
Gradient elution	Initial conditions 80% H_2O and 25% ACN, ACN was increasing until 50 % for 25 min and remained for 20 min, and then up to 100% ACN for 15 min
Data acquisition	Full scan (100-1000 *m/z*)
Source	Positive electrospray ionization (ESI)
Vaporizer Tª	400 °C
Capillary Tª	350 °C

Table 1: LC-MS conditions

RESULTS AND DISCUSSION

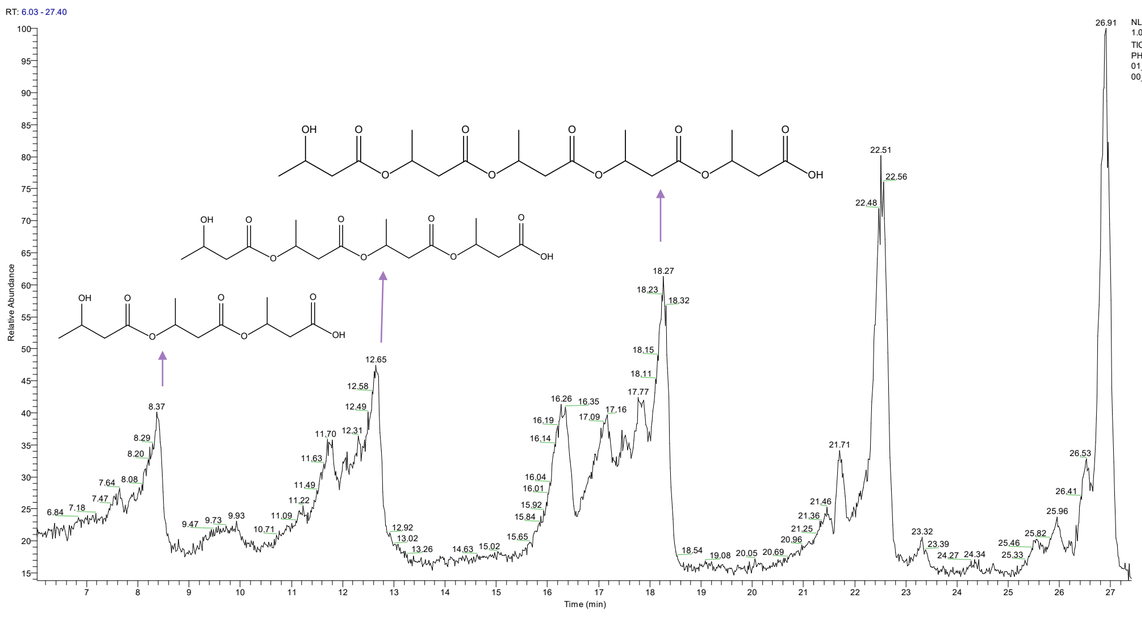


Figure 1: Chromatogram of PHB pellet extract by LC-MS

RT (min)	Proposed compound	m/z	Adduct	TC
8.37	3HB trimer	277.2, 294.2, 299.2	H^+, NH_4^+, Na^+	I
12.65	3HB tetramer	363.2, 380.2, 385.2	H^+, NH_4^+, Na^+	I
16.26	3HB cyclic/saturated trimer	259.2, 276.2, 281.2	H^+, NH_4^+, Na^+	I/I
18.27	3HB pentamer	449.2, 466.3, 471.2	H^+, NH_4^+, Na^+	I
21.89	3HB hexamer	535.3, 552.3, 557.3	H^+, NH_4^+, Na^+	III
22.51	3HB cyclic/saturated tetramer	345.2, 362.2, 367.2	H^+, NH_4^+, Na^+	I/I
25.70	3HB heptamer	621.3, 638.3, 643.3, 659.2	H^+, NH_4^+, Na^+, K^+	III
26.91	3HB cyclic/saturated pentamer	431.2, 448.3, 453.2	H^+, NH_4^+, Na^+	I/I
28.30	3HB octamer	707.3, 724.4, 729.3 745.3,	H^+, NH_4^+, Na^+, K^+	III
29.70	3HB cyclic/saturated hexamer	517.3, 534.3, 539.3, 555.2	H^+, NH_4^+, Na^+, K^+	I/III
30.46	3HB nonamer	810.4, 815.3, 831.3	NH_4^+, Na^+, K^+	III
33.50	3HB cyclic/saturated heptamer	620.3, 625.3	NH_4^+, Na^+	I/III
34.31	3HB decamer	896.4, 901.4	NH_4^+, Na^+	III
38.10	3HB undecamer	982.6, 987.4	NH_4^+, Na^+	III
38.51	3HB cyclic/saturated octamer	706.3, 711.3, 727.2	NH_4^+, Na^+, K^+	I/III
45.43	3HB cyclic/saturated nonamer	792.4, 797.3	NH_4^+, Na^+	I/III
50.72	3HB cyclic/saturated decamer	878.3, 883.4	NH_4^+, Na^+	I/III
52.86	3HB cyclic/saturated undecamer	964.4, 969.4, 985.4	NH_4^+, Na^+, K^+	I/III

Table 2: Tentative identified PHB oligomers in the ACN extracts. RT: retention time; 3HB: 3-hydroxybutyrate; TC: level of toxicity according to Cramer rules

CONCLUSIONS

Several PHB derivatives were tentatively identified for the first time, according to our knowledge, by comparing their *m/z* characteristics with a homemade database developed taking into account possible starting substances, both linear and cyclic forms.

Cramer's decision tree was used to estimate the toxicity of the identified compounds, since for these compounds there is still no toxicological data available nor commercial standards available and, therefore, to date no migration limits have been established. Several oligomers were classified as class III (high toxicity), so the next step would be to carry out a risk assessment of these compounds.

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