Quantification of structure-property relationships for plant polyesters reveals suberin and cutin idiosyncrasies

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Supporting Information contains 26 pages including 28 Figures and 4 Tables.

Index

analyzed by NMR: a) ^{1}H NMR in DMSO-d_6 in different time points, b) representative of ^{1}H-^{13}C HMBC NMR after 24 h of reaction. The dashed red circle mark the region of the spectrum where the signal assigned to the acid resulting from the hydrolysis would be noticed.

- **Table S1** – Elemental analysis of tomato cutin, pepper cutin, cork suberin and potato suberin. Relative percentage of Nitrogen (N), Carbon (C), Hydrogen (H) and Oxygen (O) in the different samples.
- **Table S2 –** Total carbohydrate quantification of cutin and suberin samples.
- **Figure S5** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO-d_6 at 60 ºC.
- **Figure S6 –** 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO-d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.
- **Figure S7** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S8** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S9** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.
- **Figure S10** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S11** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of cork suberin in DMSO-d_6 at 60 ºC.
- **Figure S12** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of cork suberin in DMSO- d_6 at 60 ºC.
- **Figure S13** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of cork suberin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S14** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of potato suberin in DMSO-d_6 at 60 ºC.
- **Figure S15** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of potato suberin in DMSO- d_6 at 60 ºC.
- **Figure S16** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of potato suberin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Table S3** – Monomeric composition of cutin purified from tomato or pepper using cholinium hexanoate (2 h), identified by NMR. Chemical structures and ^{1}H and ^{13}C chemical shifts (ppm) of the cutin

monomers. Signals not resolved in the NMR spectra due to overlapping signals or not yet assigned are not shown in this table. Most of the signals have been assigned before by us[1].

- **Table S4** – Monomeric composition of suberin extracted from cork or potato using cholinium hexanoate (2 h), identified by NMR. Chemical structures and ^{1}H and ^{13}C chemical shifts (ppm) of the cutin monomers. Signals not resolved in the NMR spectra due to overlapping signals or not yet assigned are not shown in this table. Most of the signals have been assigned before by us[2].
- **Figure S17** – Quantification of crystalline cellulose concentration with ImageJ software, of cutin from tomato, pepper, Micro-tom wild type and mutants purified with cholinium hexanoate. The result was expressed in mg of crystalline cellulose per mg of cutin. The error bars represent the standard deviations of three independent measurements.
- **Figure S18 -** SEM micrographs of cutin samples extracted from Micro-Tom tomatoes, wild type (a) and the mutants *cus1* (b) and *gpat6* (c), using cholinium hexanoate extraction for 2h.
- **Figure S19 -** 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S20** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.
- **Figure S21** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.
- **Figure S22** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC.
- **Figure S23** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.
- **Figure S24** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC.
- **Figure S25** – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO- d_6 at 60 ºC.
- **Figure S26** – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

- **Figure S27** – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO-d_6 at 60 ºC.
- **Figure S28** - Wide-ranging NMR spectral characterisation of Micro-tom cutins: wild-type (a), *cus1* (b) and *gpat6* (c) mutants; upon isolation with cholinium hexanoate (2 h). The ^{1}H NMR spectra of all samples (I); HSQC regions corresponding to aliphatics (II) and CH/CH2-X aliphatics (III). Some correlations (unlabeled) are uncertain or unidentified.

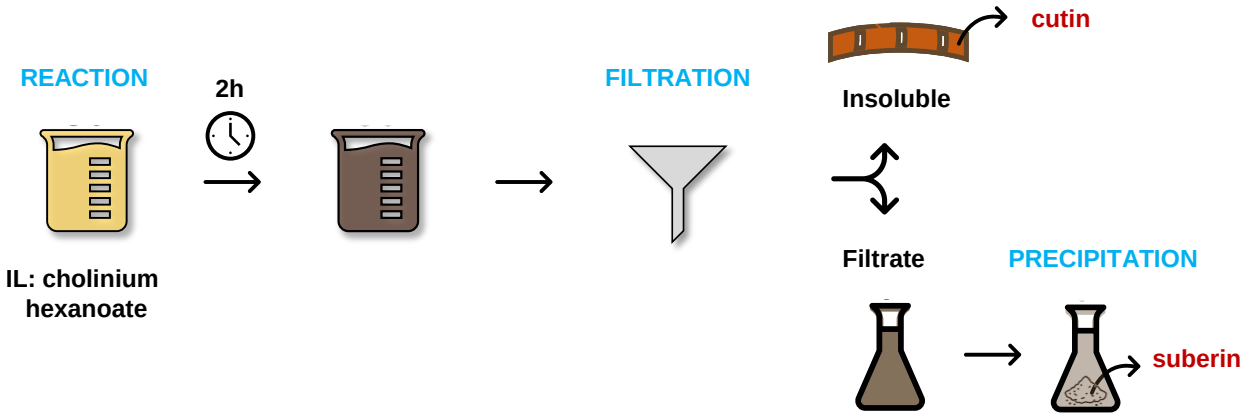


Figure S1 – Schematics of the extraction process

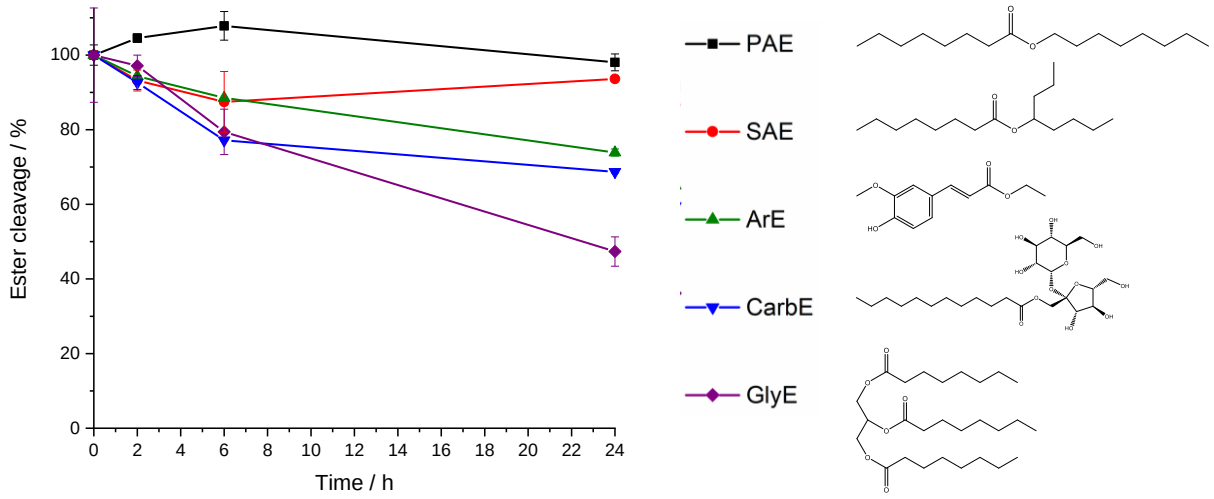


Figure S2 –The capacity of the cholinium hexanoate to mediate the hydrolysis of distinct ester-type bonds, up to 24h at 100 ºC with stirring, was analyzed by GC-MS. Standard compounds representative of five ester-types were used: a primary aliphatic ester (PAE, octyl octanoate), a secondary aliphatic ester (SAE, octan-4-yl octanoate), an aromatic ester (ArE, ethyl 4-hydroxy-3-methoxy-cinnamate), a carbohydrate ester (CarbE, sucrose monolaurate) and a glycerol ester (GlyE, glyceryl trioctanoate). PAE and GlyE kinetic curves were adapted from a dataset published before by us[1].

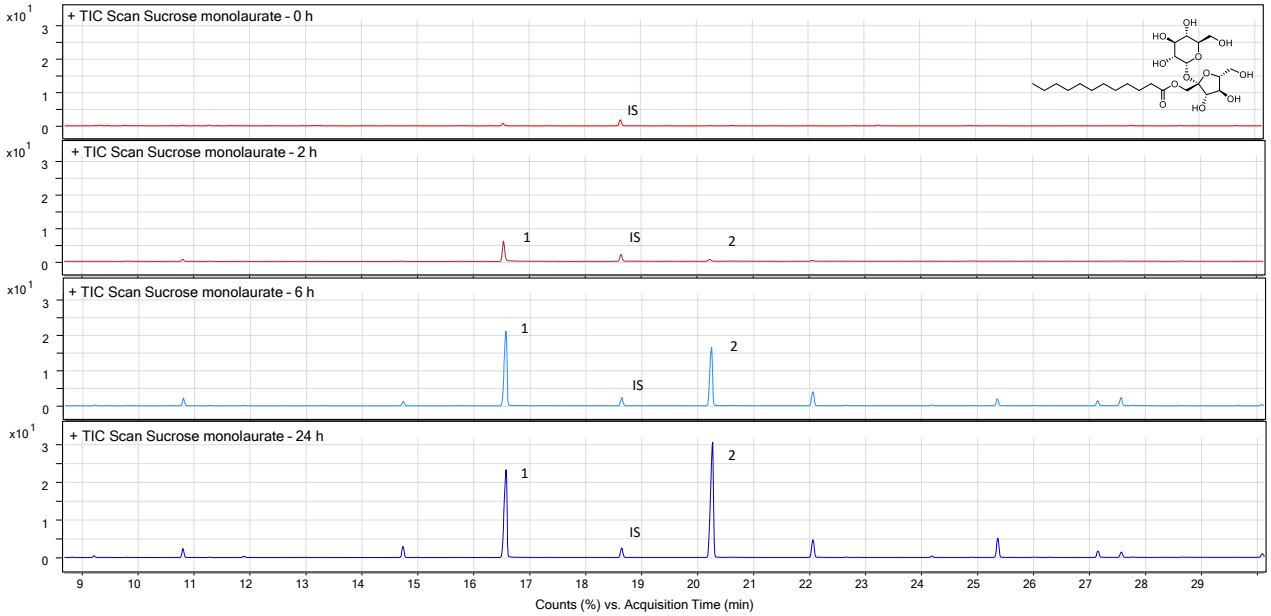


Figure S3 - Representative GC-MS chromatograms of the model compound sucrose monolaurate after reaction with cholinium hexanoate at increasing time points. Over time the peak assigned to dodecanoic acid - hydrolysis product of sucrose monolaurate, increased.

IS – internal standard (hexadecane); 1 – dodecanoic acid, methyl ester; 2 – dodecanoic acid, trimethylsilyl ester.

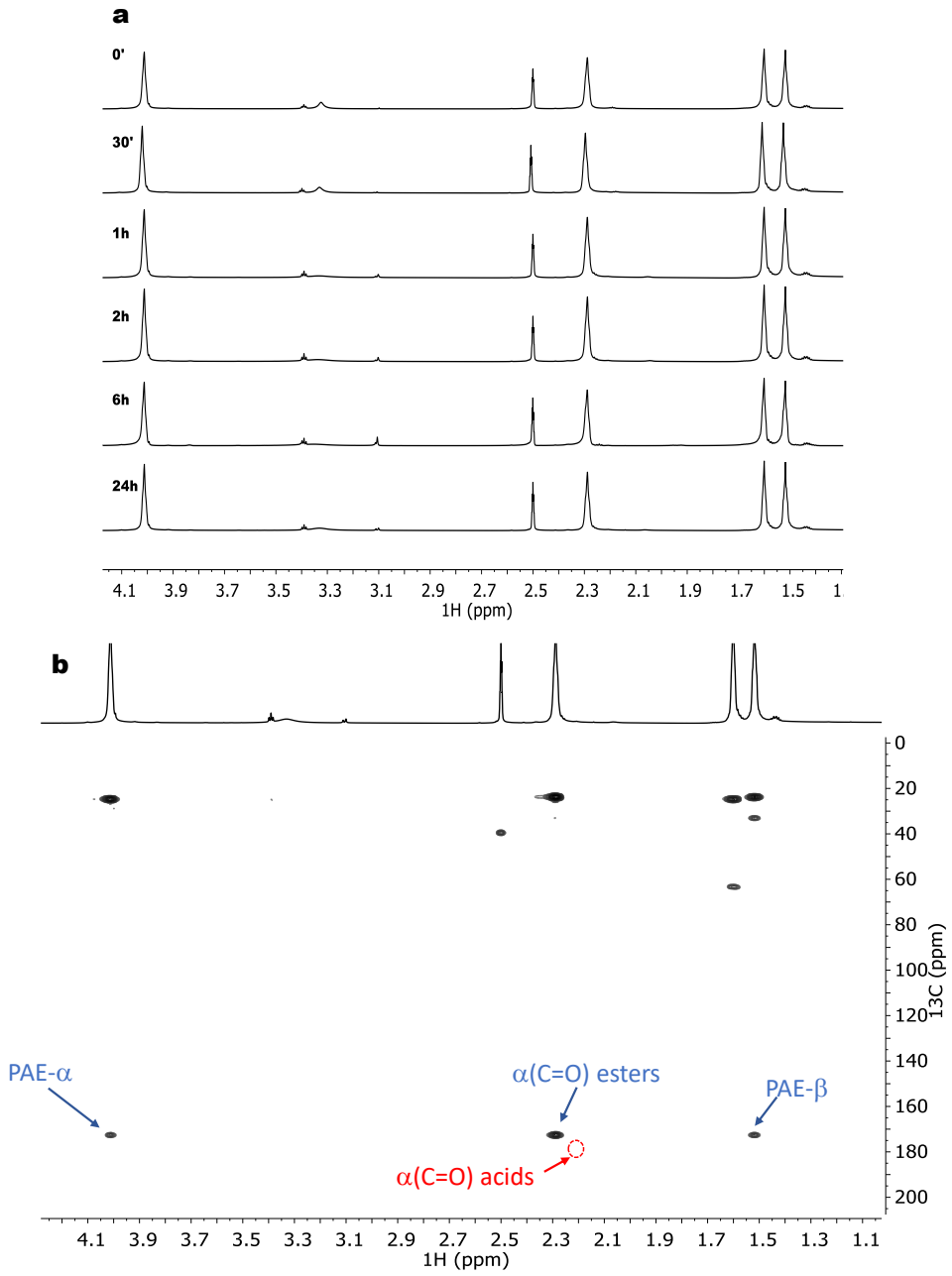


Figure S4 - The capacity of the cholinium hexanoate to mediate the hydrolysis of primary aliphatic esters bonds in a synthetic polymer, poly(1,4-butyl adipate), up to 24h at 100 ºC with stirring, was analyzed by NMR: a) ^{1}H NMR in DMSO-d_6 in different time points, b) representative of ^{1}H-^{13}C HMBC NMR after 24 h of reaction. The dashed red circle marks the region of the spectrum where the signal assigned to the acid resulting from the hydrolysis would be noticed.

Table S1 – Elemental analysis of tomato cutin, pepper cutin, cork suberin and potato suberin. Relative percentage of Nitrogen (N), Carbon (C), Hydrogen (H) and Oxygen (O) in the different samples

	Sample	Tomato Cutin	Pepper Cutin	Cork Suberin	Potato suberin
Elemental Analysis	Nitrogen (N)[a] (%)	< 1	< 1	1.60 ± 0.08	2.33 ± 0.07
	Carbon (C) (%)	63.98 ± 0.16	67.63 ± 0.02	64.07 ± 0.02	60.65 ± 0.11
	Hydrogen (H) (%)	9.08 ± 0.12	9.79 ± 0.03	8.86 ± 0.06	8.54 ± 0.07
	Oxygen (O) (%)	26.96 ± 0.27	22.60 ± 0.04	26.08 ± 0.04	28.50 ± 0.11

a) corresponding peak areas were outside the calibration curve, the suberin values may be subject to error yet shown since the monomer 4-(2-aminoethyl)phenol contains N. Deviation from theoretical percentage (should be <0.4%);

Table S2 – Total carbohydrate quantification of cutin and suberin samples.

Samples	ng (carbohydrate)/mg (plant polyester)	carbohydrate content / %
Tomato cutin	0.489 ± 0.127	$4.89E^{-5} \pm 1.27E^{-5}$
Pepper cutin	0.124 ± 0.016	$1.25E^{-5} \pm 1.79E^{-6}$
Cork suberin	0.21 ± 0.033	$2.10E^{-5} \pm 3.34E^{-6}$
Potato suberin	1.16 ± 0.31	$9.70E^{-5} \pm 5.51E^{-5}$
Micro-Tom *wt*	0.387 ± 0.072	$3.87E^{-5} \pm 7.19E^{-6}$
Micro-Tom *cus1*	1.022 ± 0.496	$1.02E^{-4} \pm 4.96E^{-5}$
Micro-Tom *gpat6*	0.541 ± 0.175	$5.53E^{-5} \pm 3.00E^{-5}$

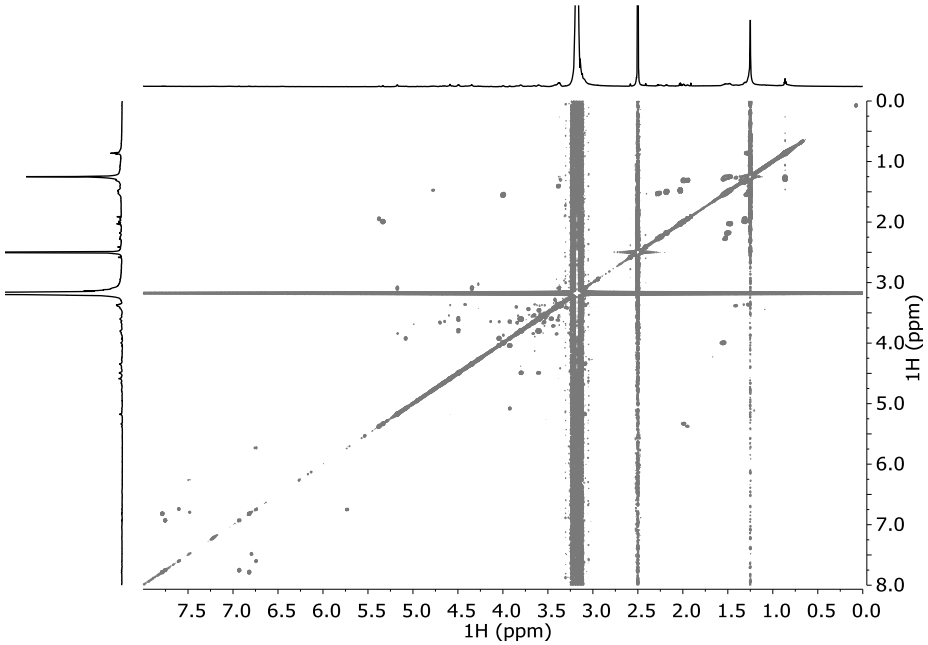


Figure S5 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO-d_6 at 60 ºC.

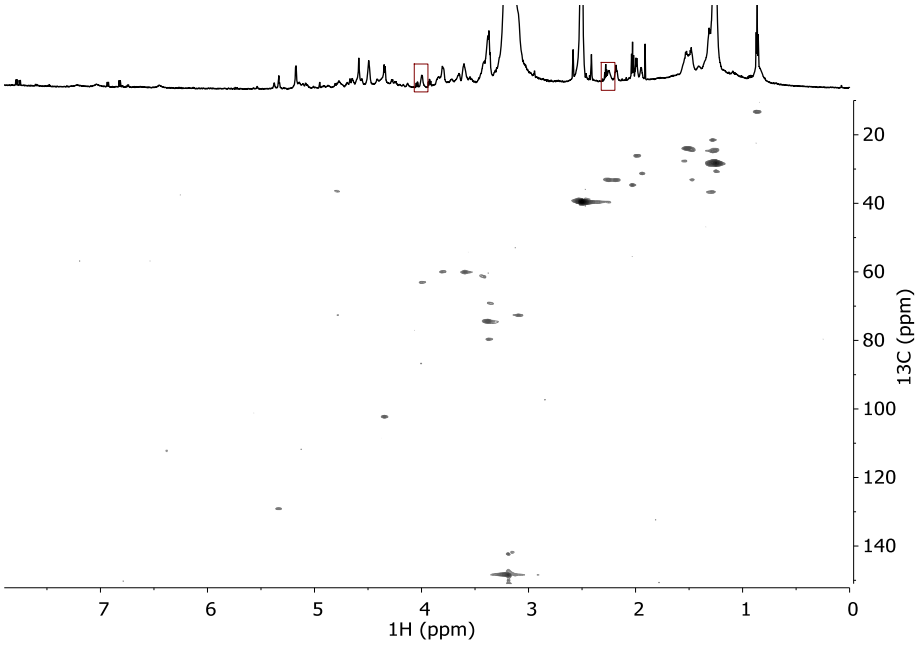


Figure S6 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO-d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

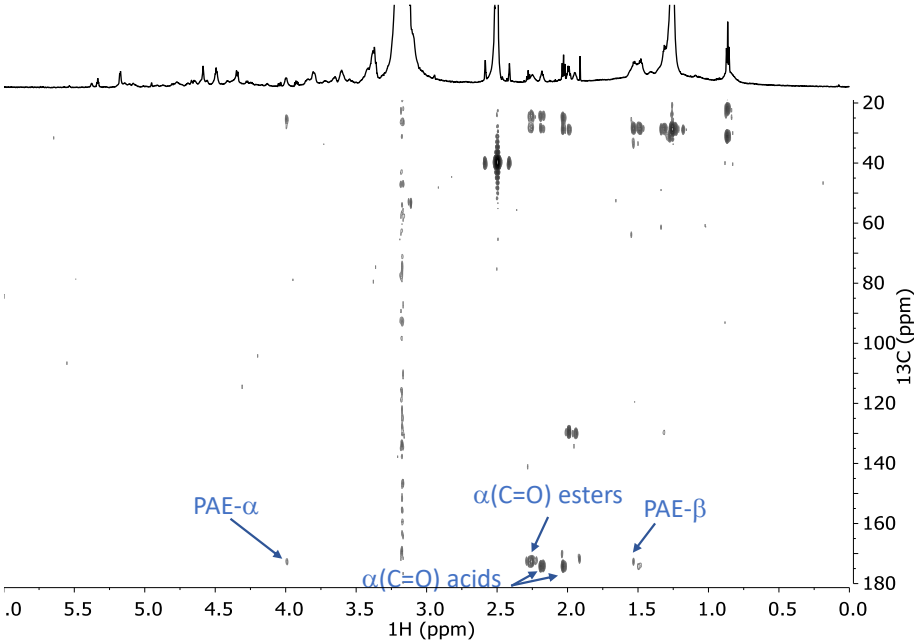


Figure S7 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of tomato cutin extracted using cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

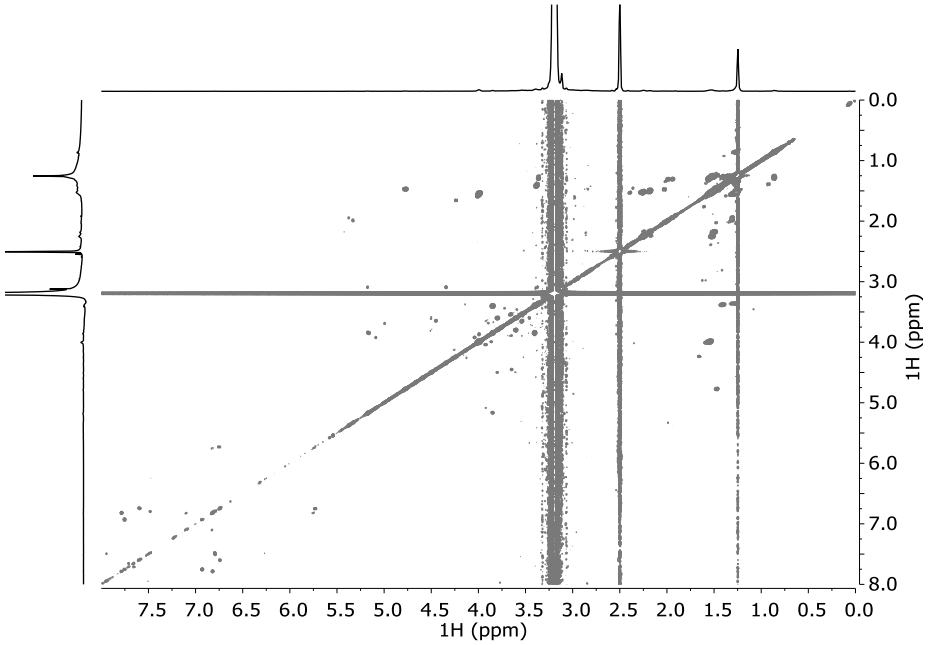


Figure S8 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

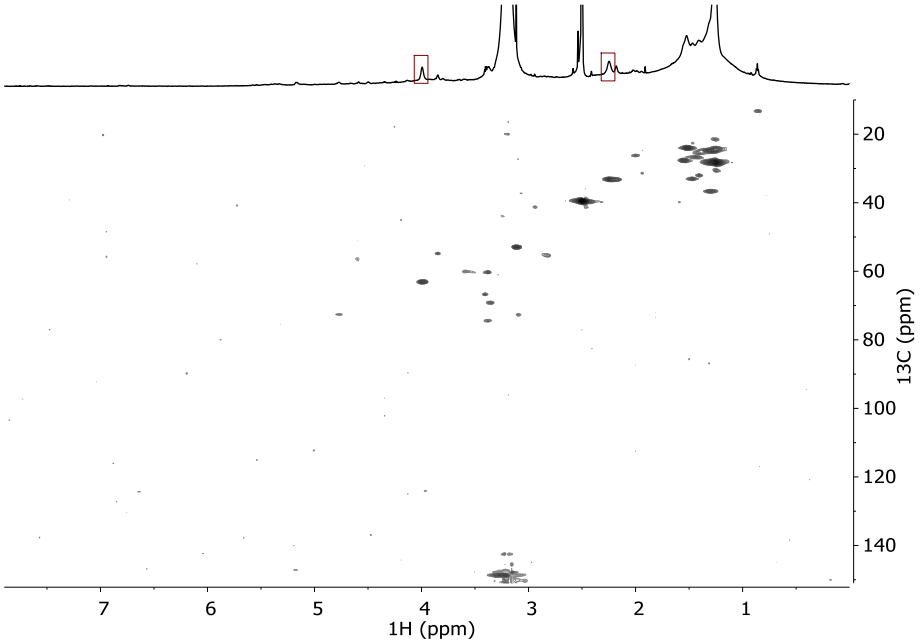


Figure S9 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

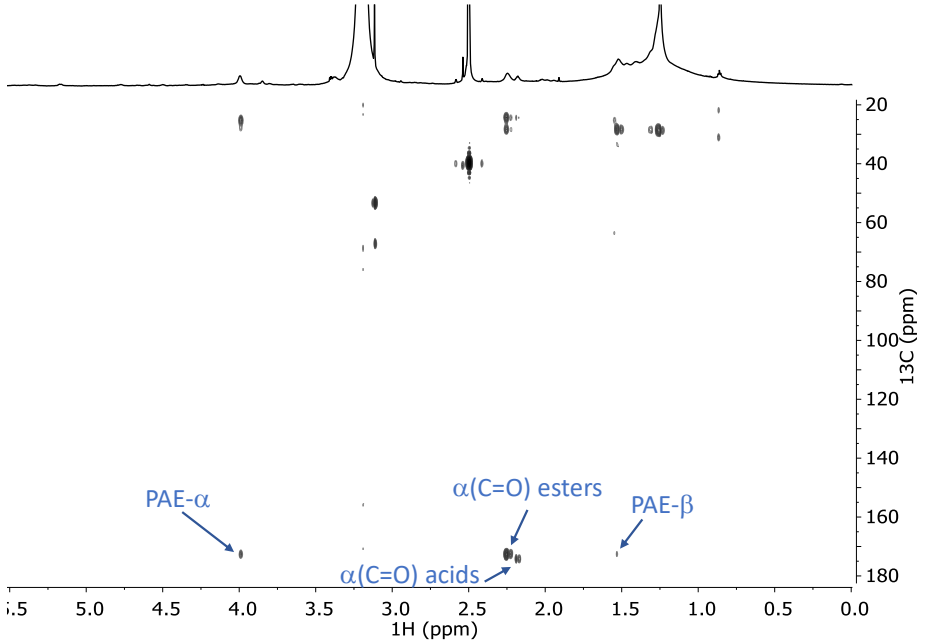


Figure S10 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of pepper cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

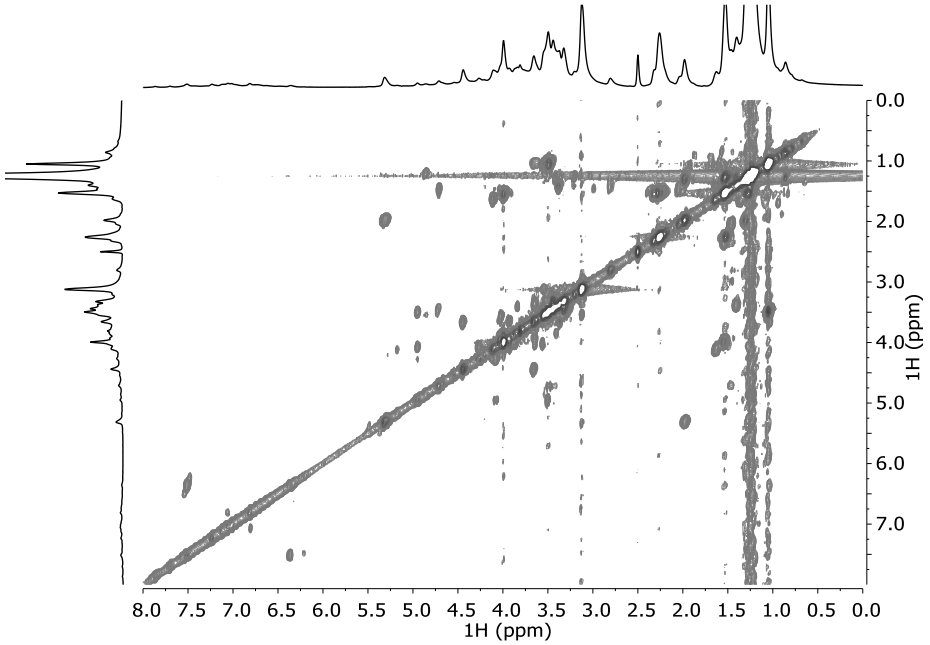


Figure S11 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of cork suberin in DMSO-d_6 at 60 ºC.

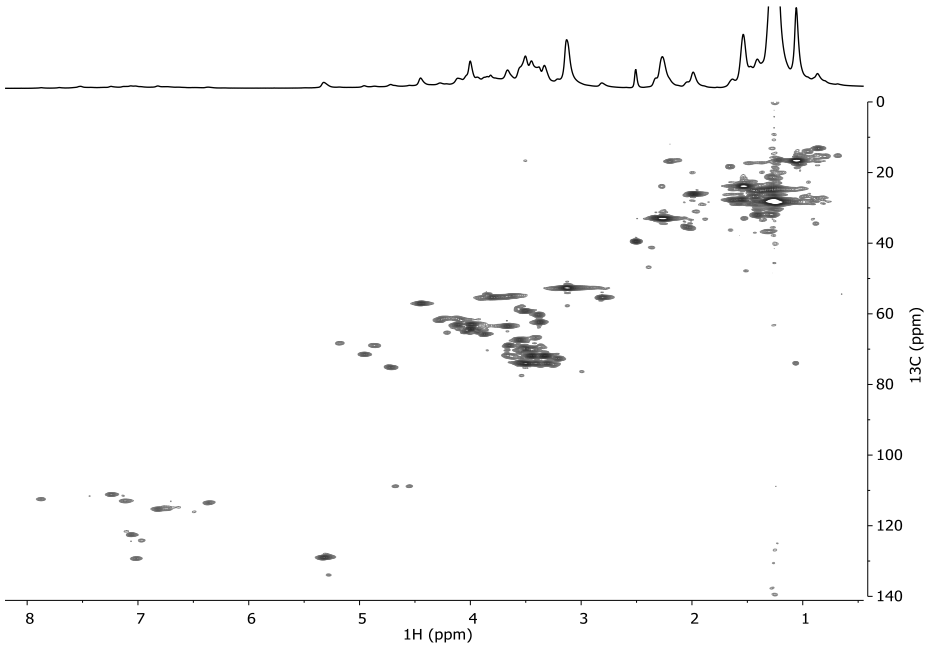


Figure S12 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of cork suberin in DMSO- d_6 at 60 ºC.

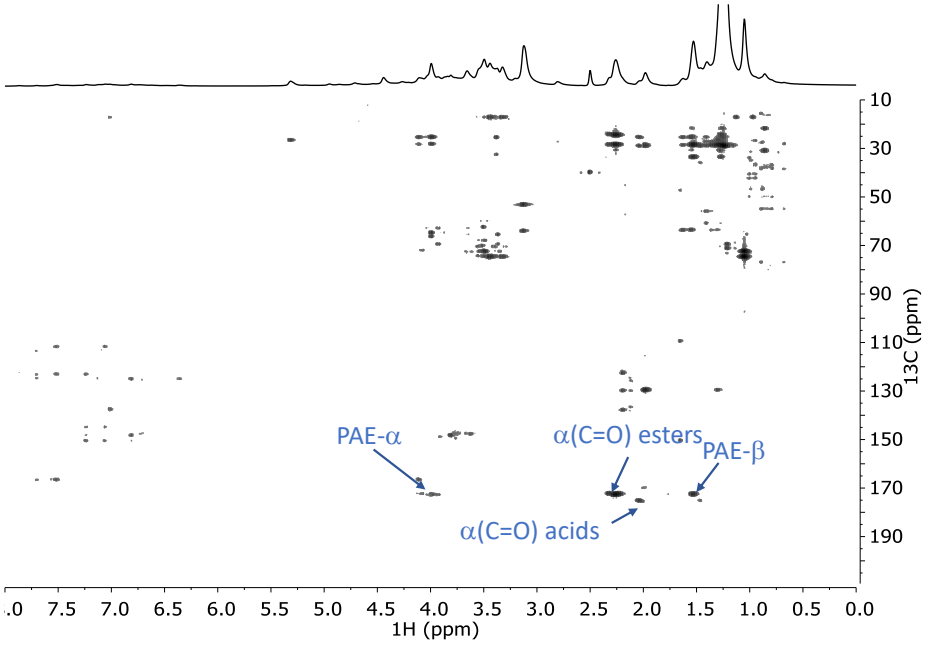


Figure S13 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of cork suberin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

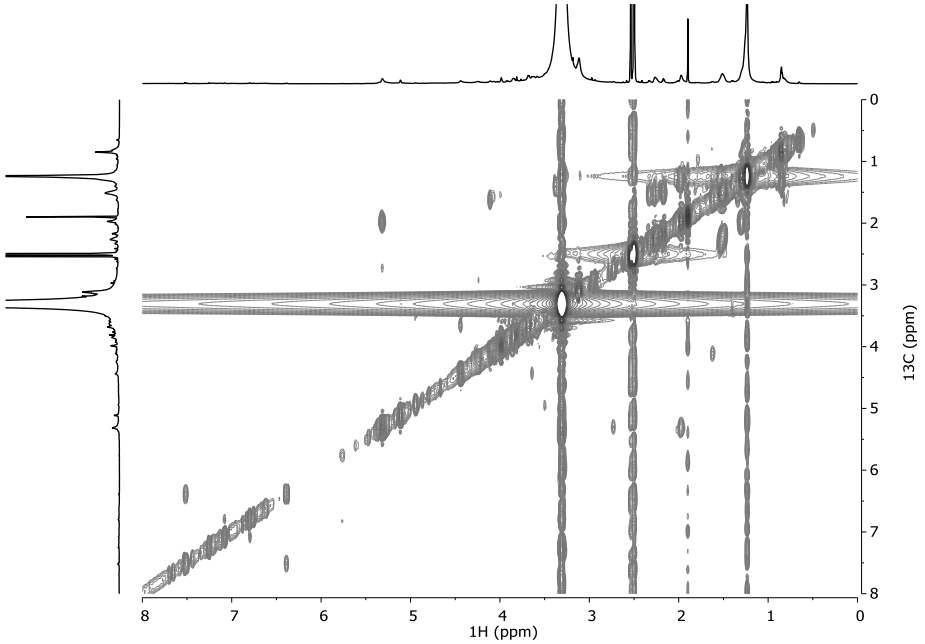


Figure S14 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of potato suberin in DMSO-d_6 at 60 ºC.

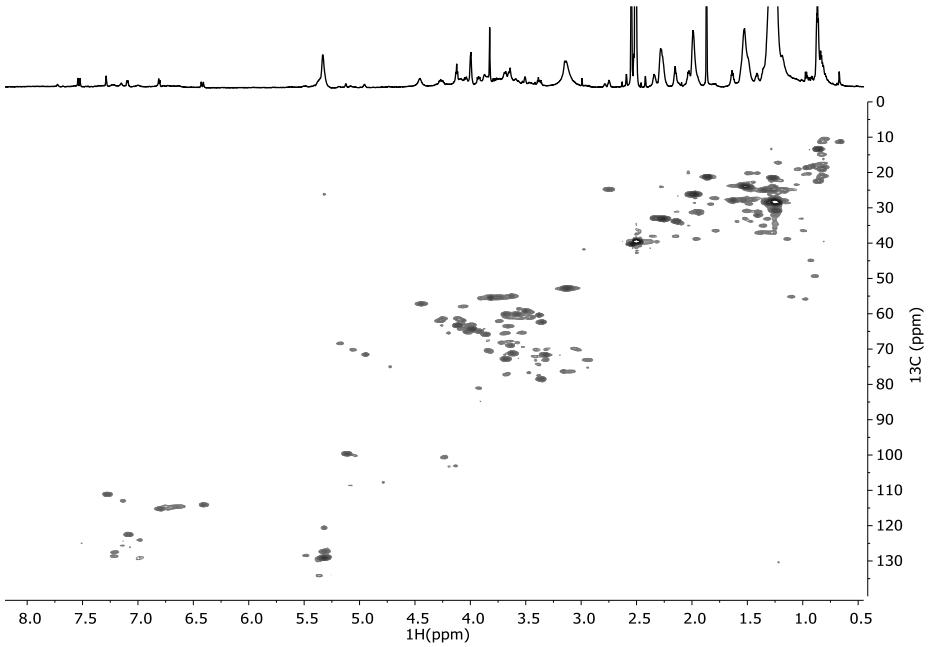


Figure S15 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of potato suberin in DMSO- d_6 at 60 ºC.

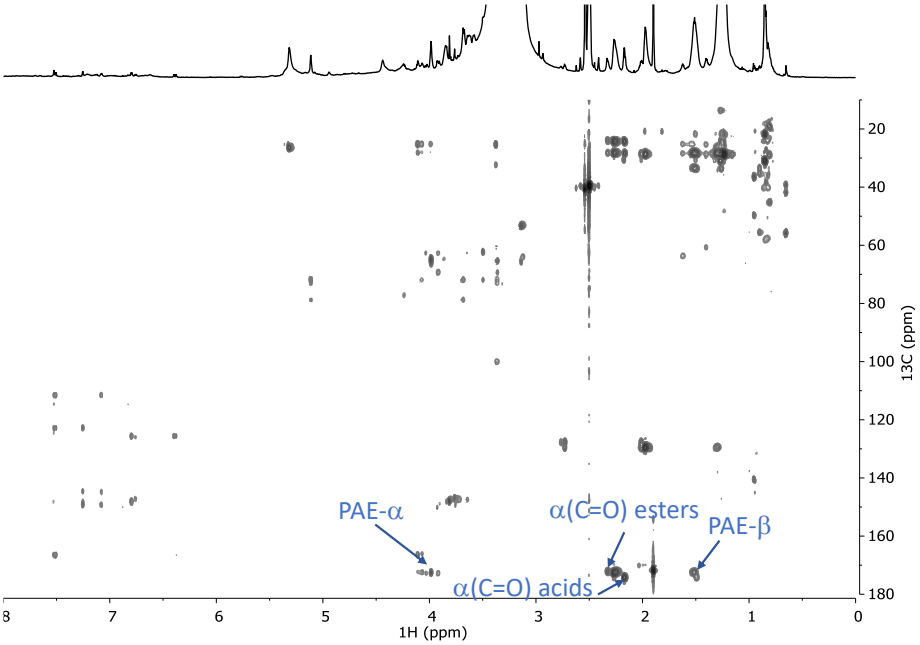
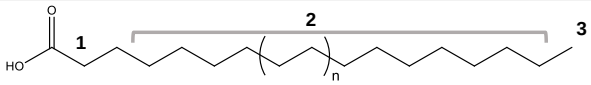
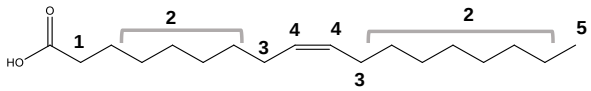
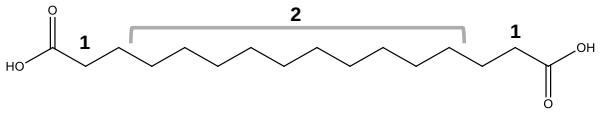


Figure S16 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of potato suberin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

Table S3 – Monomeric composition of cutin purified from tomato or pepper using cholinium hexanoate (2 h), identified by NMR. Chemical structures and ^{1}H and ^{13}C chemical shifts (ppm) of the cutin monomers. Signals not resolved in the NMR spectra due to overlapping signals or not yet assigned are not shown in this table. Most of the signals have been assigned before by us[1].

Chemical structure	Chemical shifts (ppm)
Fatty acids (n= 1-2)	
	^{1}H NMR (800.33 MHz, DMSO-d_6) δ 0.86 (m, 3H, H-3), 1.25 (m, 2H, H-2), 2.02 (m, 2H, H-1). ^{13}C NMR (201.42 MHz, DMSO-d6) δ 13.39 (C-3), 28.34 (C-2), 34.67 (C-1).
	^{1}H NMR (800.33 MHz, DMSO-d6) δ 0.86 (m, 3H, H-5), 1.25 (m, 2H, H-2), 1.99 (m, 2H, H-3), 2.02 (m, 2H, H-1), 5.34 (m, 2H, H-4). ^{13}C NMR (201.42 MHz, DMSO-d6) δ 13.39 (C-5), 26.13 (C-3), 28.34 (C-2), 34.67 (C-1), 129.07 (C-4).
Dicarboxylic Acids	
	^{1}H NMR (800.33 MHz, DMSO-d6) δ 1.25 (m, 2H, H-2), 2.02 (m, 2H, H-1).

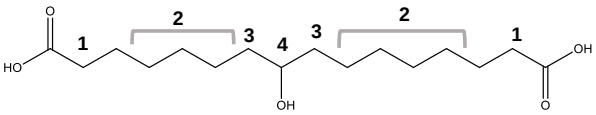
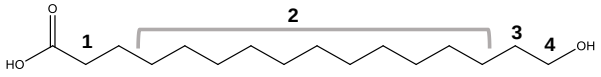
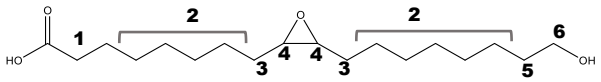
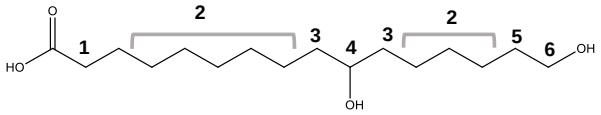
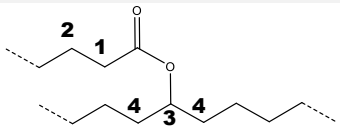
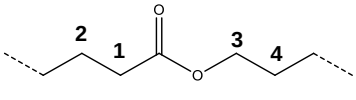
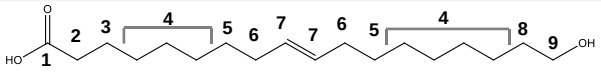
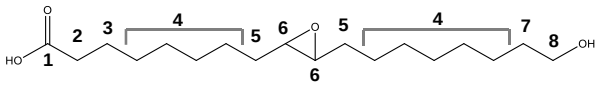
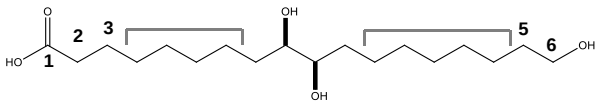
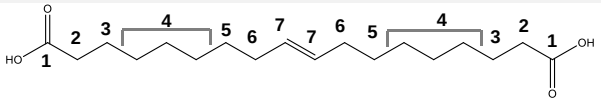
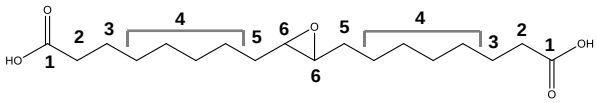
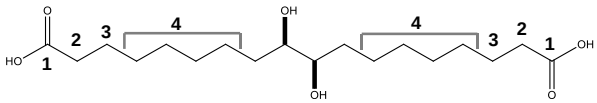
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 28.34 (C-2), 34.67 (C-1).
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.25 (m, 2H, H-2), 1.30 (m, 2H, H-3), 2.02 (m, 2H, H-1), 3.38 (m, 2H, H-4). ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 28.34 (C-2), 34.67 (C-1), 36.64 (C-3), 74.31 (C-4).
ω-hydroxyacids	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.25 (m, 2H, H-2), 1.41 (m, 2H, H-3), 2.02 (m, 2H, H-1), 3.41 (m, 2H, H-4). ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 28.34 (C-2), 32.03 (C-3), 34.67 (C-1), 67.64 (C-4).
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.25 (m, 2H, H-2), 1.42 (m, 2H, H-5), 1.41 (m, 2H, H-5), 1.43 (m, 2H, H-3), 2.02 (m, 2H, H-1), 2.82 (m, 2H, H-4), 3.41 (m, 2H, H-6) ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.68 (C-3), 28.34 (C-2), 32.03 (C-5) 34.67 (C-1), 55.43 (C-4), 67.64 (C-6)
Polyhydroxyacids	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.25 (m, 2H, H-2), 1.30 (m, 2H, H-3), 1.41 (m, 2H, H-5), 2.02 (m, 2H, H-1), 3.38 (m, 2H, H-4), 3.41 (m, 2H, H-6). ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 28.34 (C-2), 32.03 (C-5), 36.64 (C-3), 34.67 (C-1), 67.64 (C-6), 74.31 (C-4).
SECONDARY ALIPHATIC ESTERS	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.47 (m, 2H, H-4), 1.54 (m, 2H, H-2), 2.25 (m, 2H, H-1), 4.77 (m, 2H, H-3). ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 27.60 (C-2), 33.05 (C-4), 33.27 (C-1), 72.60 (C-3).
PRIMARY ALIPHATIC ESTERS	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.49 (m, 2H, H-4), 1.54 (m, 2H, H-2), 2.25 (m, 2H, H-1), 3.98 (m, 2H, H-3). ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 24.02 (C-4), 27.60 (C-2), 33.27 (C-1), 63.05 (C-3).

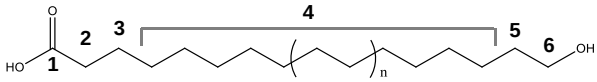
Table S4 – Monomeric composition of suberin extracted from cork or potato using cholinium hexanoate (2 h), identified by NMR. Chemical structures and ^{1}H and ^{13}C chemical shifts (ppm) of the cutin monomers. Signals not resolved in the NMR spectra due to overlapping signals or not yet assigned are not shown in this table. Most of the signals have been assigned before by us[2].

Chemical structure	Chemical shifts (ppm)
C18 MID-CHAIN MODIFIED	
ω-hydroxyacids	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.41 (m, 2H, H-5), 1.41 (m, 2H, H-8), 1.46 (m, 2H, H-3), 1.98 (m, 2H, H-6) 2.03 (m, 2H, H-2), 3.39 (m, 2H, H-9), 5.31 (m, 2H, H-7) ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 27.07 (C-6), 29.86 (C-5), 29.35 (C-4), 32.95 (C-8), 36.40 (C-2), 61.32 (C-9), 129.98 (C-7), 172.20 (C-1)
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.41 (m, 2H, H-7), 1.43 (m, 2H, H-5), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2), 2.80 (m, 2H, H-6), 3.39 (m, 2H, H-8) ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 27.83 (C-5), 32.95 (C-7), 36.40 (C-2), 56.38 (C-6), 61.32 (C-8), 172.20 (C-1)
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.41 (m, 2H, H-5), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2), 3.39 (m, 2H, H-6) ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 32.95 (C-5), 36.40 (C-2), 61.32 (C-6), 172.20 (C-1)
α,ω-diacids	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.41 (m, 2H, H-5), 1.46 (m, 2H, H-3), 1.98 (m, 2H, H-6), 2.03 (m, 2H, H-2), 5.31 (m, 2H, H-7)

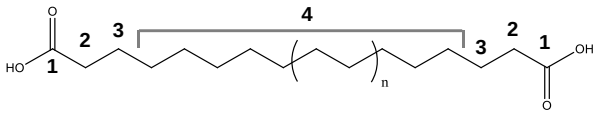
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 27.07 (C-6), 29.86 (C-5), 29.35 (C-4), 36.40 (C-2), 129.98 (C-7), 172.20 (C-1)
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.43 (m, 2H, H-5), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2), 2.80 (m, 2H, H-6)
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 27.83 (C-5), 36.40 (C-2), 56.38 (C-6), 172.20 (C-1)
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2)
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 36.40 (C-2), 172.20 (C-1)

SATURATED CHAIN

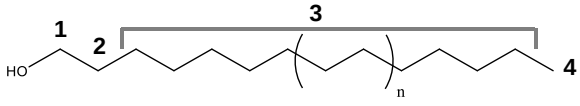
ω-hydroxyacids (n= 1-5)

	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.41 (m, 2H, H-5), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2), 3.39 (m, 2H, H-6)
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 32.95 (C-5), 36.40 (C-2), 61.32 (C-6), 172.20 (C-1)

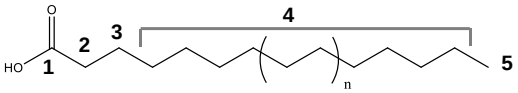
α,ω-diacids (n= 1-5)

	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.24 (m, 2H, H-4), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2)
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 26.13 (C-3), 29.35 (C-4), 36.40 (C-2), 172.20 (C-1)

Fatty alcohols (n= 1-5)

	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 0.86 (m, 3H, H-4), 1.24 (m, 2H, H-3), 1.41 (m, 2H, H-1), 3.39 (m, 2H, H-2)
	^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 14.13 (C-4), 29.35 (C-3), 32.95 (C-2), 61.32 (C-1)

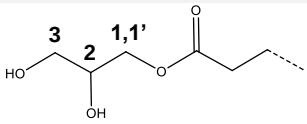
Fatty acids (n= 1-5)



^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 0.86 (m, 3H, H-5), 1.24 (m, 2H, H-4), 1.46 (m, 2H, H-3), 2.03 (m, 2H, H-2)
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 14.13 (C-5), 26.13 (C-3), 29.35 (C-4), 36.40 (C-2), 172.20 (C-1)

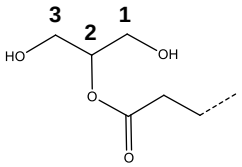
ACYLGLYCEROL ESTERS

1-monoacylglycerol (1-MAG)



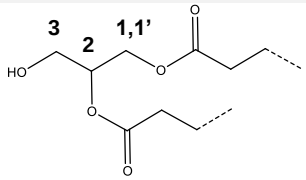
^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 3.37 (m, 2H, H-3), 3.65 (m, 1H, H-2), 3.93 (m, 1H, H-1), 4.03 (m, 1H, H-1')
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 63.35 (C-3), 65.88 (C1,1'), 69.96 (C-2)

2-monoacylglycerol (2-MAG)



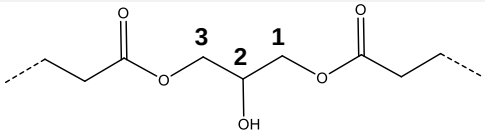
^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 3.50 (m, 2H, H-1,3), 4.71 (m, 1H, H-2)
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 60.17(C-1,3), 76.13 (C-2)

1,2-diacylglycerol (1,2-DAG)



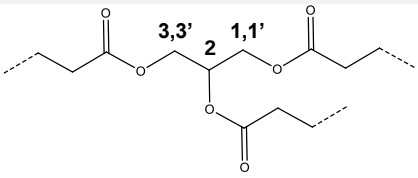
^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 3.50 (m, 2H, H-3), 4.08 (m, 2H, H-1), 4.27 (m, 2H, H-1'), 4.95 (m, 1H, H-2)
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 60.17 (C-3), 62.76 (C-1'), 62.81 (C-1), 72.46 (C-2)

1,3-diacylglycerol (1,3-DAG)

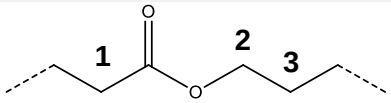


^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 3.87 (m, 1H, H-2), 3.99 (m, 4H, H-1,3)
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 65.25 (C-1,3), 66.72 (C-2)

1,2,3-triacylglycerol (1,2,3-TAG)



^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 4.12 (m, 2H, H-1,3),4.24 (m, 2H, H-1',3'), 5.17 (m, 1H, H-2)
^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 62.33 (C1'-3'), 62.40 (C1-3), 69.32 (C2)

PRIMARY ALIPHATIC ESTERS	
	^{1}H NMR (800.33 MHz, DMSO-*d*6) δ 1.53 (m, 2H, H-3), 2.26 (m, 2H, H-1), 3.99 (m, 2H, H-2) ^{13}C NMR (201.42 MHz, DMSO-*d*6) δ 24.85 (C-3), 34.03 (C-1), 64.02 (C-2)

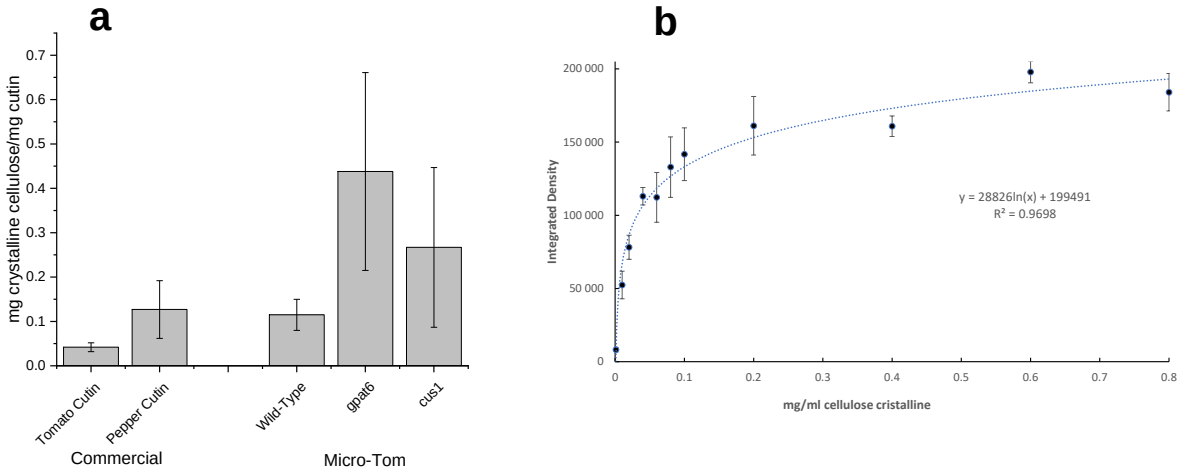


Figure S17 – Quantification of crystalline cellulose concentration with ImageJ software of cutin from tomato, pepper and Micro-tom (wild type and mutants) purified with cholinium hexanoate. The result was expressed in mg of crystalline cellulose per mg of cutin in sample (a). The error bars represent the standard deviations of three independent measurements. Standard calibration curve for quantitative detection of crystalline cellulose based on direct dot-blot immunoassay using Image J software (b). The error bars represent the standard deviations of three independent measurements.

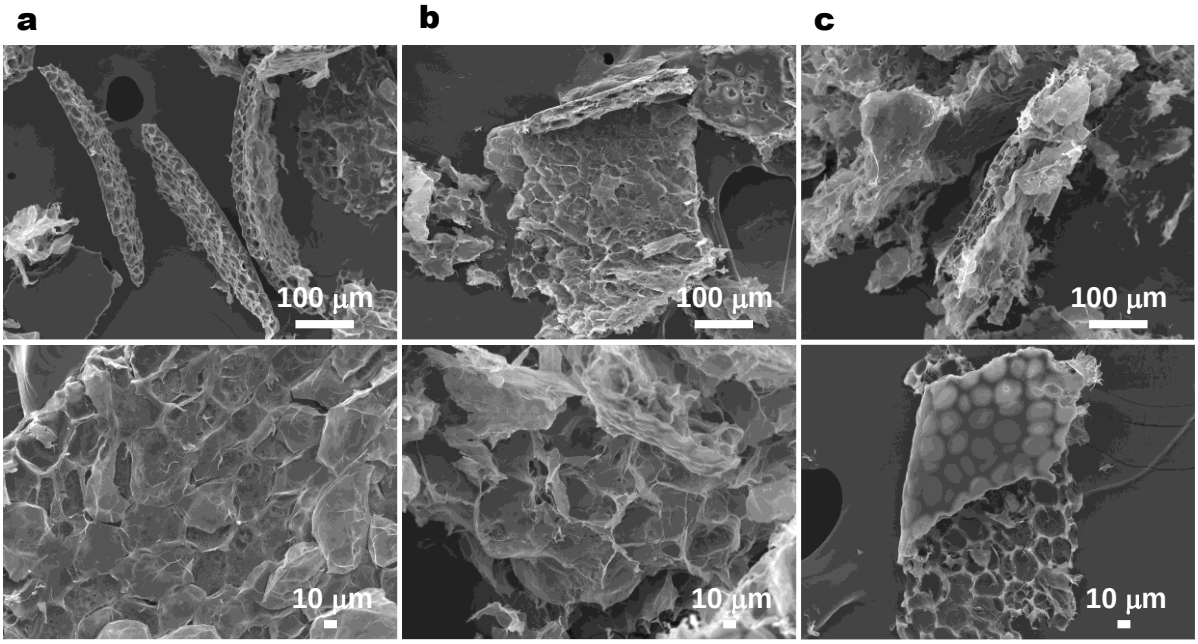


Figure S18 - SEM micrographs of cutin samples extracted from Micro-Tom tomatoes, wild type (a) and the mutants *cus1* (b) and *gpat6* (c), using cholinium hexanoate extraction for 2h.

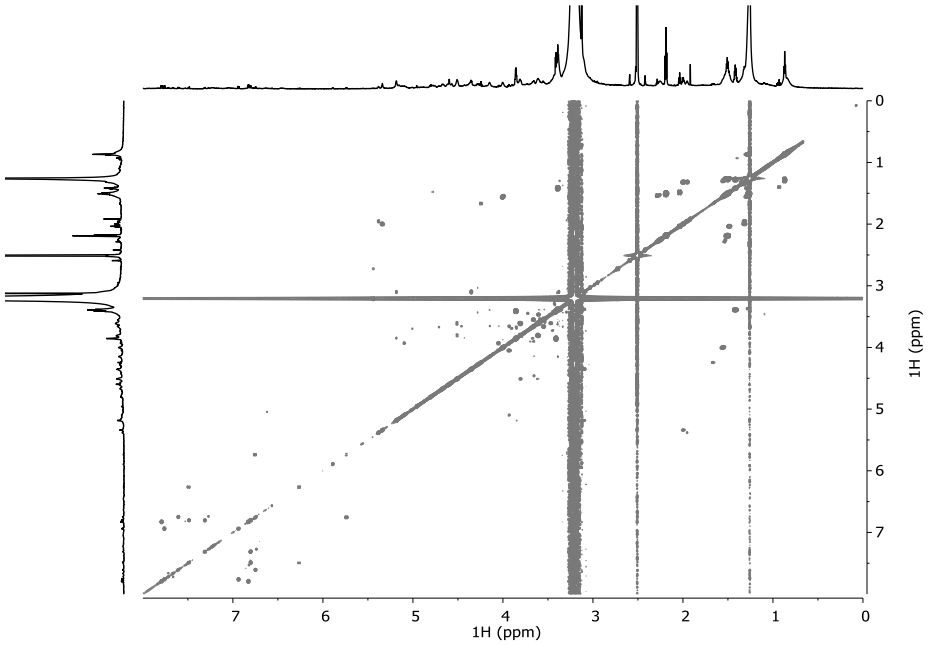


Figure S19 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO-d_6 at 60 ºC.

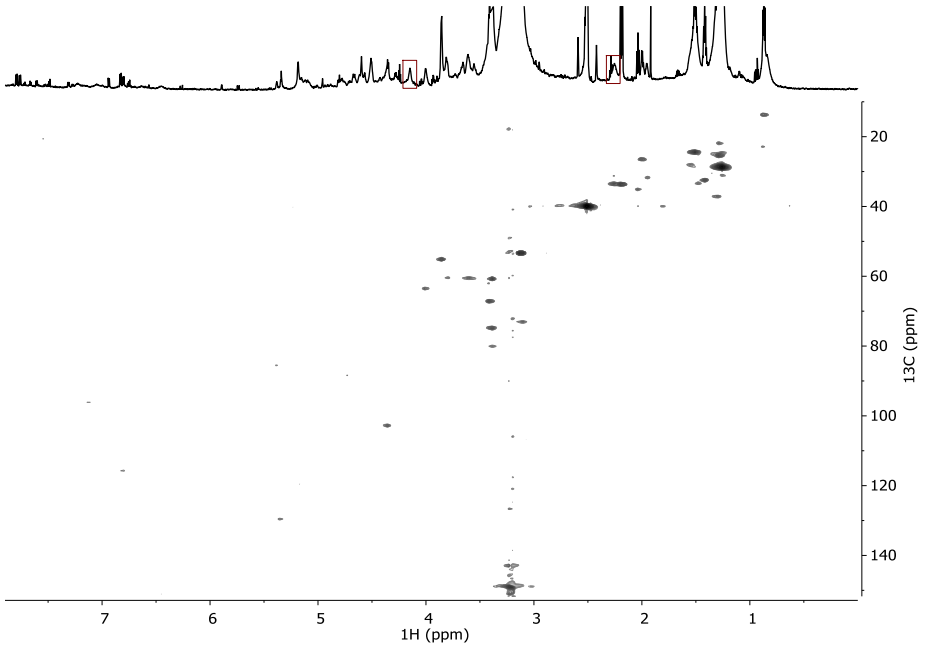


Figure S20 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

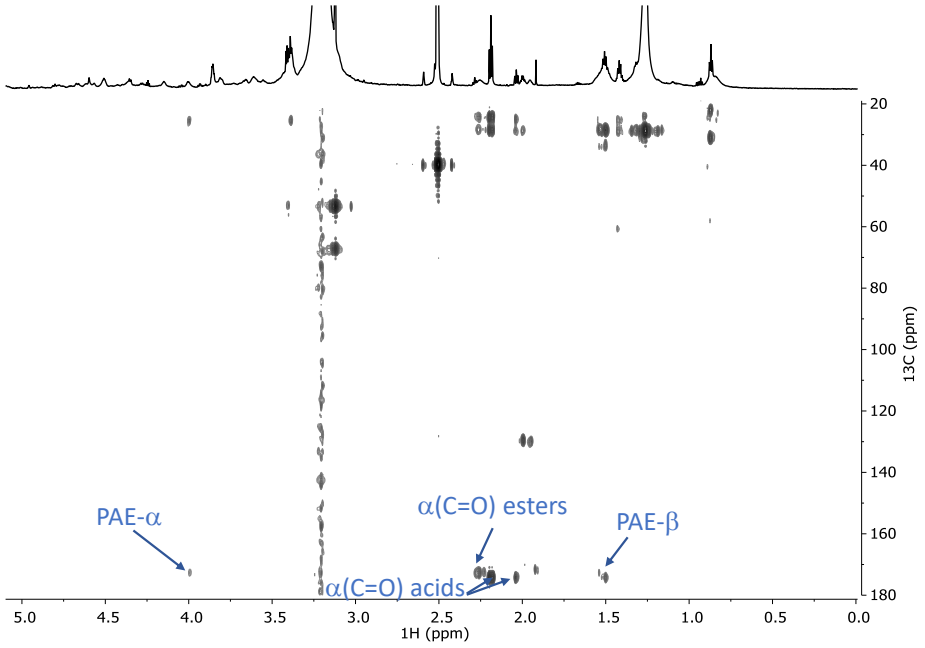


Figure S21 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) in DMSO- d_6 at 60 ºC.

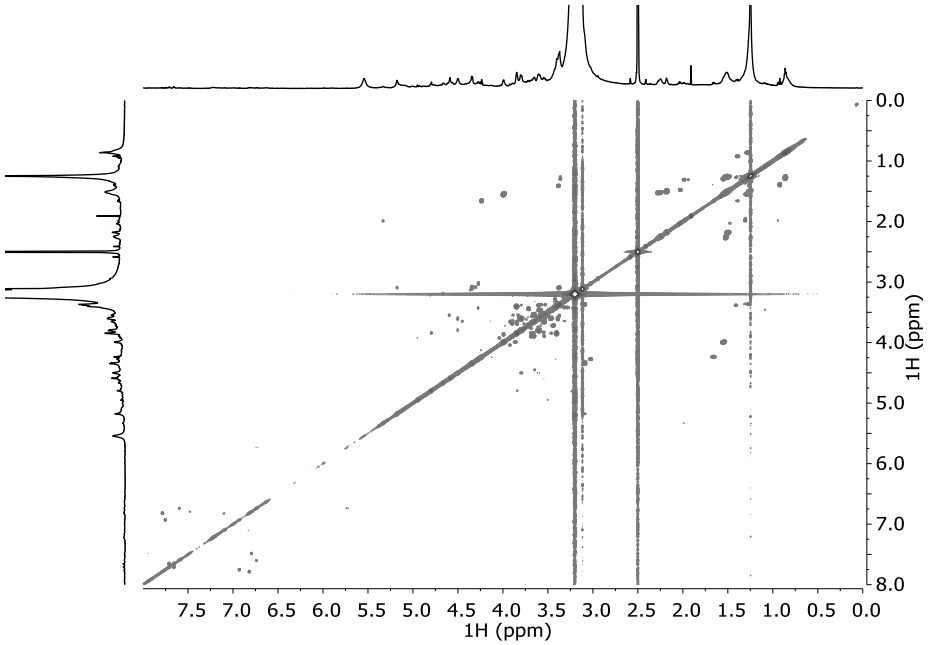


Figure S22 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC.

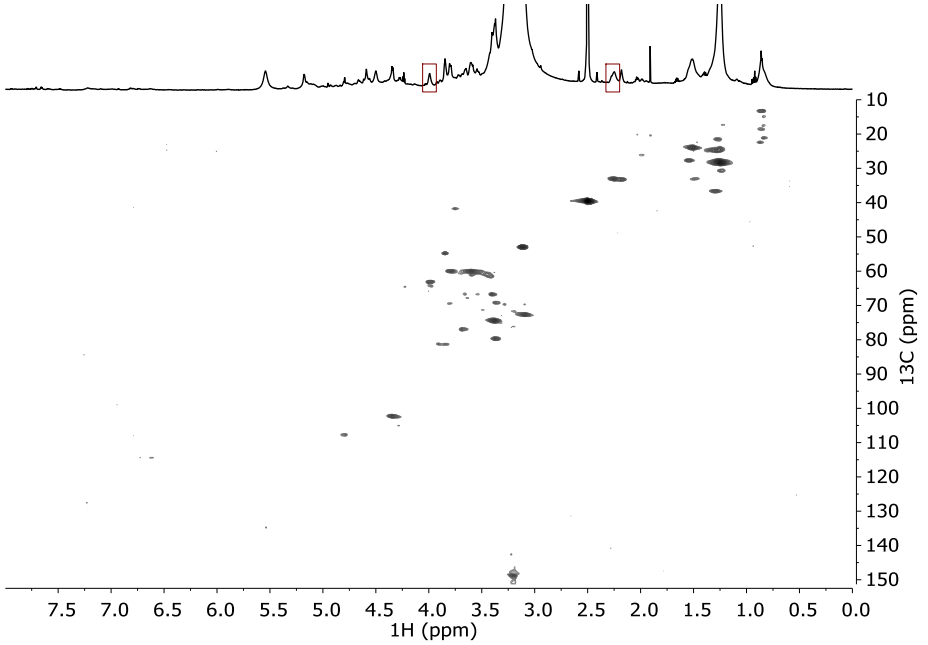


Figure S23 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

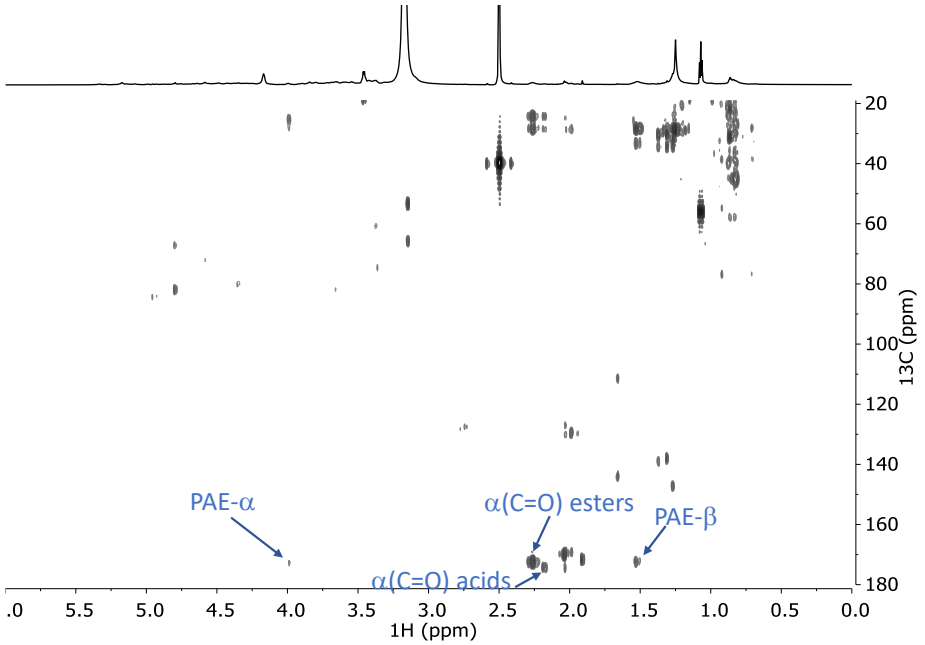


Figure S24 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *cus1* mutant in DMSO- d_6 at 60 ºC.

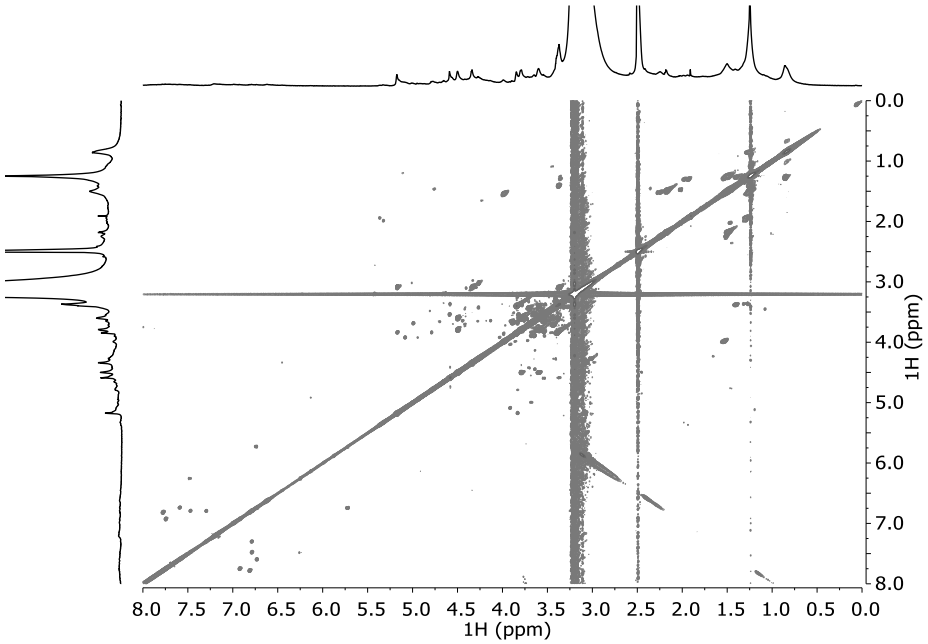


Figure S25 – 2D-^{1}H-^{1}H COSY-NMR spectrum (COrrelated SpectroscopY) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO- d_6 at 60 ºC.

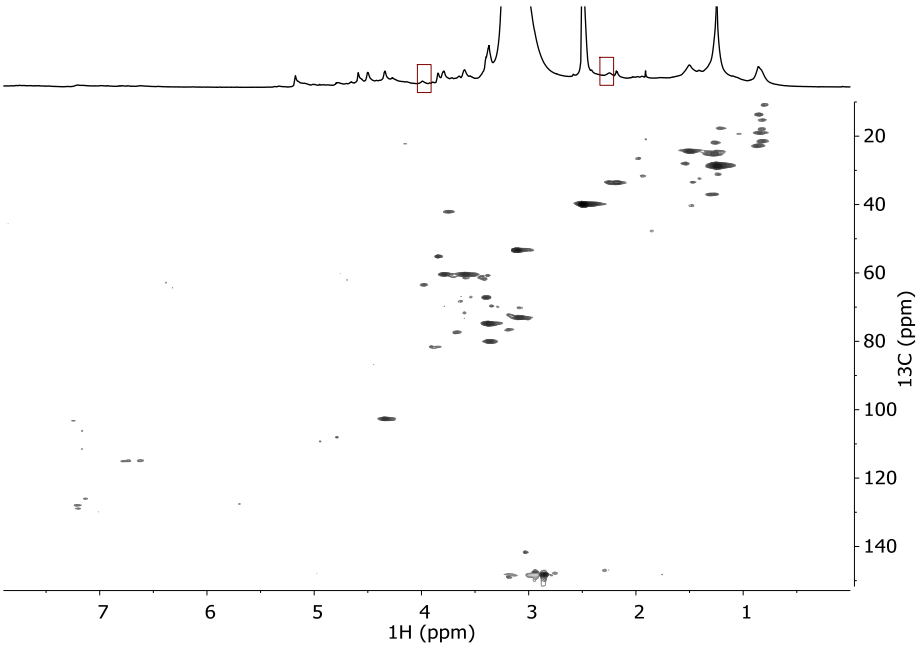


Figure S26 – 2D-^{1}H-^{13}C HSQC-NMR spectrum (Heteronuclear Single Quantum Coherence) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO- d_6 at 60 ºC. The red rectangles mark signals used for the integration of specific functional groups, all of which showed no overlap of resonances with other chemical groups as shown by the bi-dimensional NMR correlations done.

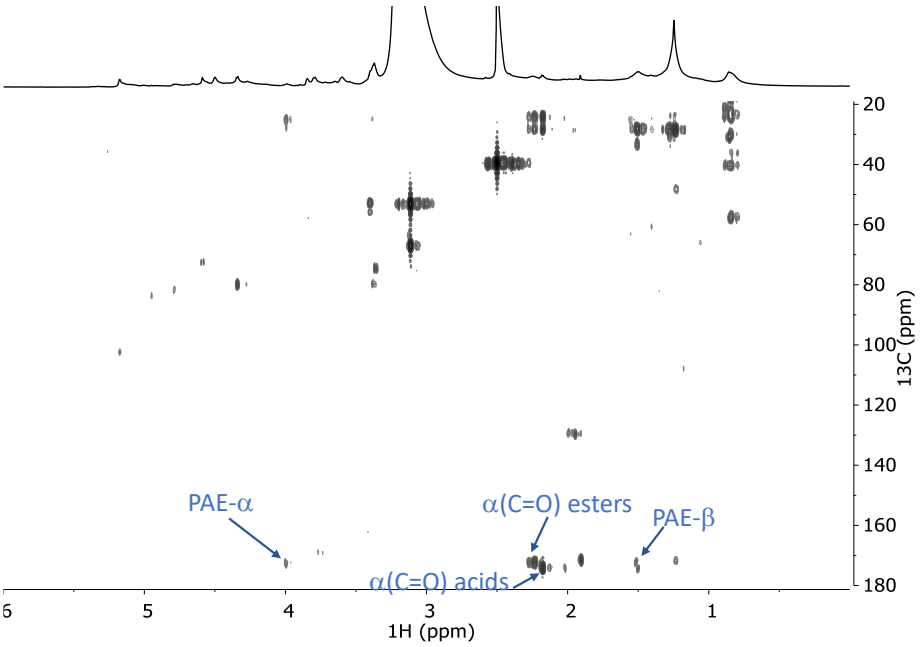


Figure S27 – 2D-^{1}H-^{13}C HMBC-NMR spectrum (Heteronuclear Multiple Bond Correlation) of Micro-Tom cutin isolated with cholinium hexanoate (2 h) from the *gpat6* mutant in DMSO-d_6 at 60 ºC.

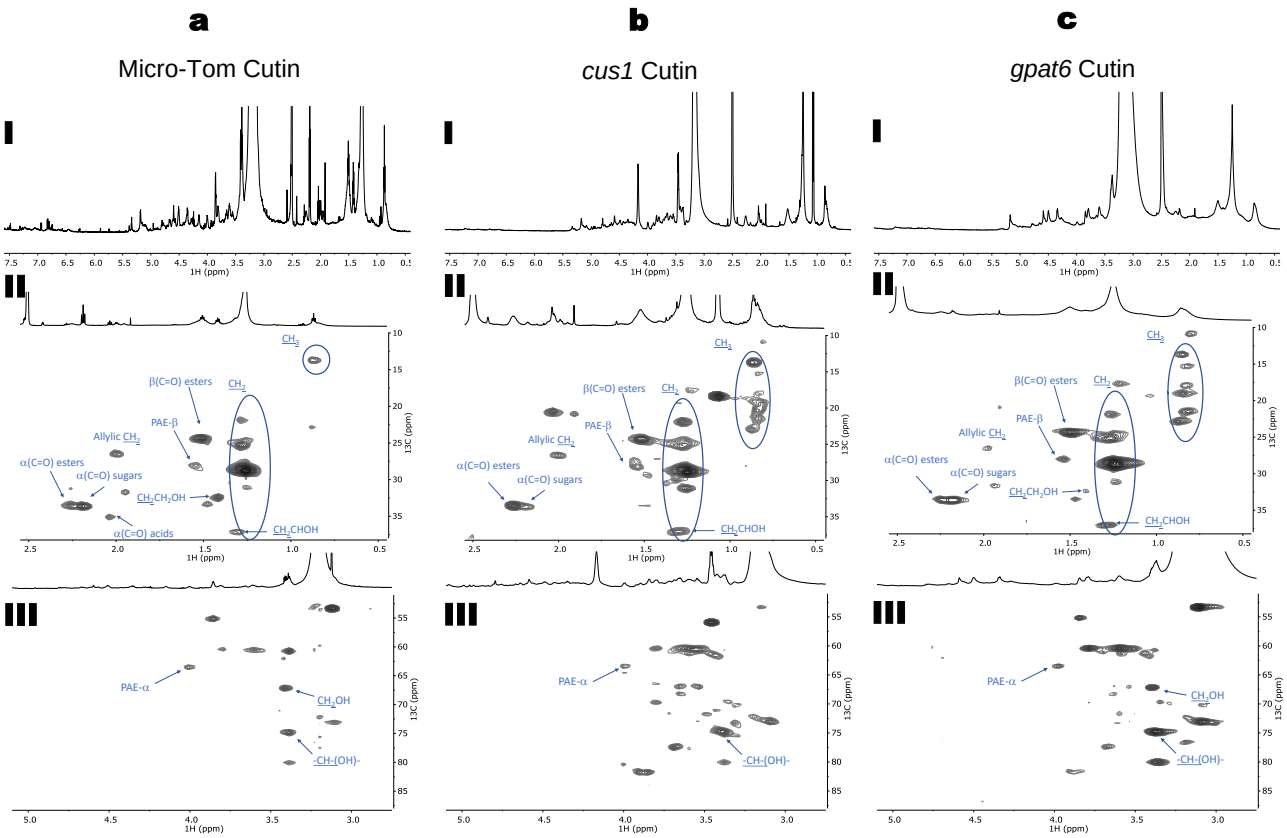


Figure S28 - Wide-ranging NMR spectral characterisation of Micro-tom cutins: wild-type (a), *cus1* (b) and *gpat6* (c) mutants; upon isolation with cholinium hexanoate (2 h). The ^{1}H NMR spectra of all samples (I); HSQC regions corresponding to aliphatics (II) and CH/CH2-X aliphatics (III). Some correlations (unlabelled) are uncertain or unidentified.

References

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