Supplementary Material:

Opuntisines, 14-membered cyclopeptide alkaloids from fruits of *Opuntia stricta* var. *dillenii* isolated by high-performance countercurrent chromatography

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Fruit Analysis: Detection of Opuntisines by LC-ESI-MS in *Opuntia stricta* var. *dillenii* tissues.

Chromatographic description and spectroscopic data of minor concentrated cyclopeptide alkaloids from *Opuntia stricta* var. *dillenii*

Efficiency and characterization of HPCCC separation of Opuntisines - LC-ESI-MS/MS data of unknown Opuntisine cyclopeptide metabolites

The superior separation efficiency of preparative HPCCC for cyclopeptide alkaloids is seen in the results of very low chromatographic resolution of LC-ESI-MS analysis (cf. Suppl. Fig. S1) of the phase layer evaluation experiments. Specifically for the target ion $[M+H]^+$ at m/z 620, only two of the isobaric opuntisines were separated by analytical C18-chromatography. Applying the *off-line* ESI-MS injection approach where the preparative results of HPCCC separation were projected (cf. Fig. 1), 4 areas of isobaric metabolites (**1. 620-a, 2. 620-b and 620-c, 3. 620-d and 620-e, 4. 620-f**) were visualized by the changes in m/z 620 ion intensities of the gaussian shaped elution profile. The two-dimensional chromatography approach using LC-ESI-MS analysis securely detected in total 6 opuntisine A isobars. The results on some analysed HPCCC fractions (F33, F36, F42, F48, F54, F56 and F62) are displayed in Fig. 3a and Fig. 3b showing that the C₁₈-LC-elution times of the separated very minor concentrated isobars are slightly different (R_t 33-35 min). In this specific case, HPCCC had demonstrated an excellent separation capacity to fractionate metabolite isobars (diastereomers) of $[M+H]^+$ m/z 620.

The dehydroxy-derivative opuntisine B (**2**) with $[M+H]^+$ at m/z 604 was recovered in the final extruded fractions of the HPCCC experiment. The missing OH-function at C-1 drastically increased the lipophilic character by strong affinity to the stationary organic CCC-phase (CCC- K_D -value above 64). Predicted K_D values of co-eluting opuntisine A isobars (**620-a – 620-f**), and opuntisine B (**2**) were determined by LC-ESI-MS and are displayed in Suppl. Fig. S1.

Although, the isobars were nicely separated by HPCCC (CCC- K_D range 1.16 – 14) (cf. Fig. 1), the LC-ESI-MS experiment could only determine the K_D -mean value of all co-eluting opuntisine A derivatives (LC- K_D 1.32).

In certain cases, K_D -prediction by LC is not guiding to accurate values which are comparable to a real CCC experiment. LC-evaluation of metabolite distributions in CCC-solvent phases are normally using lower concentrations levels of valuable crude extracts than later applied to the real preparative CCC-fractionation. This fact is highly influencing the K_D -values as physicochemical effects in solution such as hydrogen-bonding might strongly alter results. This effect of dimerization with polarity masking of carboxyl-groups was already observed by Nernst in case of benzoic acid in two phase systems (Nernst, 1891) and later during CCC loading studies on *Hippophaë rhamnoides* extracts containing the phenolic acid protocatechuic acid (Gutzeit, Winterhalter & Jerz, 2007).

Overall, the elution sequence by of **1** (**620-a**), and **2** achieved by HPCCC was correctly predicted by LC-ESI-MS evaluation (cf. Suppl. Table S1).

References:

- Gutzeit, D., Winterhalter, P., & Jerz, G. (2007). Application of preparative high-speed counter-current chromatography/electrospray ionization mass spectrometry for a fast screening and fractionation of polyphenols. *Journal of Chromatography A*, 1172, 40-46.
- W. Nernst. (1891), Verteilung eines Stoffes zwischen zwei Lösemitteln und zwischen Lösemittel und Dampfraum. *Z. Phys. Chem.* 8, 110-139.

Opuntisine A isomers (isobars) and unknown cyclopeptide alkaloid metabolites

Finally, the off-line 2D-chromatographic approach (HPCCC x LC-ESI-MS/MS) revealed the existence of very minor concentrated isobars of opuntisine A (**1**) (cf. Fig. 3a). As proof for the

molecular weights of all detected cyclopeptide isobars and unknown metabolites LC-ESI-MS in the negative ionization mode was done on specific HPLC fractions (data cf. sections below).

Opuntisine A isomers (isobars) Mr 619

Interestingly, solely the isobars of **620-a** and **620-b** (opuntisine A, **1**) with $[M+H]^+$ at m/z 620 revealed the MS/MS fragmentation to m/z 341. MS/MS of **620-c** and **620-d** led to m/z 343, and m/z 359 for **620-f**, respectively (Fig. 3b). In common for all of the isobars was the base-peak fragment ion at m/z 602 with the neutral loss difference $\Delta m/z$ 18, suggesting the cleavage of the hydroxy-functionality at C-1. A typical fragment ion for **620-a** and **620-b** (**1**) was m/z 489, and m/z 507 for the **620-b** (**1**) until **620-f** (cf. Fig. 3b). Unfortunately, the recovered amounts and purities of the fractions containing these minor concentrated metabolite isobars were not sufficient for further NMR-spectroscopical analysis. Very minor concentrated m/z 620 isobars were **620-c** and **620-f** (cf. Fig. 3a).

The isobars **620-c** and **620-f** resulted in a similar MS/MS-fragmentation pattern with a base peak ion at m/z 359.9 suggesting a similar constitution. Although, both components were recovered with a large elution volume difference (approx. ΔV_R 120 mL) from the respective HPLC fractions, the two compounds revealed a very small retention time difference (ΔR_t approx. 1 min) on C_{18} -LC (cf. Fig. 3a). This was an excellent example for the complimentary separation characteristics of CCC-methodology resulting in a highly efficient separation for potentially very similar cyclopeptide alkaloid structures. Molecular weights of all isobars (**620-a** to **620-f**) with Mr 619 were ascertained by abundant $[M-H]^-$ signals at m/z 618 using LC-ESI-MS in the negative ion mode. However, the MS/MS fragment ion pattern in the negative ion mode for the observed target molecules was only in some parts consistent. Concordant ion signals revealed different ion intensities. As additional indicator for a variation

in the molecular constitutions or stereochemical situation in the six isobars, the base peak ion signals were different. As options for a structural variation in the isobars, we see a variation of amino acids units such as the exchange of isoleucin against leucin, and a *1R*-configuration of the secondary alcohol in the styrylamine unit. Also a *cis*-configuration in the hydroxy-prolin unit with 8S/9R-configuration could be discussed.

Opuntisine Mr 647 (metabolite 648)

A minor concentrated opuntisine was detected at smaller elution volumes (CCC- K_D : 1.2-1.7) (Fig. 1) indicating a more polar compound from the cyclopeptide class. The even-number molecular ion signal $[M+H]^+$ at m/z 648, and the MS/MS-fragment ion m/z 342 was identical to **620-a** and **620-b** (**1**) suggested a more polar metabolite but structurally similar cyclopeptide.

Opuntisines (metabolites 602-a, 602-b, 602-c)

In the screening of fractions from HPCCC, the ESI-MS injection profile in the positive ionization mode detected two chromatographic sections with the ion signal $[M+H]^+$ at m/z 602 (cf. Fig. 1). LC-ESI-MS analysis of respective HPCCC fractions revealed that the lower elution volume area (K_D 1.16-1.58) presented two cyclopeptide alkaloids whereas metabolite **602-a** (Rt 35.6 min) resulted in the $[M-H]^-$ signal at m/z 600, and therefore the *Mr* 601 was confirmed. The second signal (**602-b**, Rt 36.2 min) was not ionized, and therefore the *Mr* was tentative. In case of **620-c** recovered from HPCCC (K_D 3.05-4.65), a single molecule was detected in LC-ESI-MS (neg. mode) with $[M-H]^-$ at m/z 618 and also the respective ion signal $[M-H+Cl]^-$ at m/z 654/656 (Rt 33.4 min) indicating the real molecular weight *Mr* 619, whereas a strong in-source fragmentation in the positive mode might cause the neutral loss of water from an existing hydroxyl-function.

In case of **602-c** we tentatively suggest the dehydro-form of opuntisine B (**2**) with a double bond at C-1/2. This could be indicated by MS/MS fragment ions at m/z 584, 489 and 342 which

are $\Delta m/z$ 2 mass units lower than observed for **2** (Fig. 1). However, the lipophilic character of **602-c** on HPCCC (CCC- K_D : 2.8-2.9) was not as strong as observed for opuntisine B (**2**) (Fig. 1). Unfortunately the fraction amount was not sufficient to perform NMR analysis.

Opuntisines Mr 689 (metabolites 690-a/ 690-b)

In the ESI-MS profile (pos. mode) of HPCCC fractions two recovery sections for the selected ion trace $[M+H]^+$ at m/z 690 (CCC- K_D **690-a**: 0.73-0.95; **690-b**: 3.82-14) were observed (Fig. 1). The MS/MS fragment spectra of the isobars were completely different, but **690-b** displayed the identical base peak m/z 406, as already observed for metabolite **656** (Fig. 1).

Opuntisine Mr 655 (metabolite 656)

This cyclopeptide metabolite with $[M+H]^+$ at m/z 656 had shown a similar elution K_D -range (CCC- K_D : 2.8-3.8) as **602-c**. The base peak MS/MS fragment ion was m/z 406 and identical to **690-b** (Fig. 1). The indicative MS/MS-fragment ions in the positive ESI ion mode as seen for opuntisine A and B were absent.

Spectroscopical data of unknown Opuntisine cyclopeptide alkaloid metabolites (cf. Fig. 1, Fig. 3a/3b)

Opuntisine (metabolite 648, Mr 647):

HPCCC F28-F38 (V_R 140-190 mL, K_D range: 1.16 – 1.69), LC-ESI-MS (altern. +/-) from HPCCC fraction 36 (R_t 33.9 min), $[M-H]^-$: m/z 646.3, MS/MS: m/z 602 (10), 535.2 (**100**), 440.0 (20), 387.9 (20); off-line ESI-MS (+) from HPCCC $[M+H]^+$: m/z 648.3, MS/MS: m/z 630.3, 535.1, 455.0, 387.9, 341.9, 323.9, 185.9.

Isobars/ isomers of Opuntisine A (metabolites 620-a, 620-c – 620-f) characterized by LC-ESI-MS/MS (Fig 3a and 3b):

620-a (Mr 619): LC-ESI-MS (altern. +/-) from HPCCC fraction F33 (*Rt* 33.0 min), [M-H]⁻ : *m/z* 618.3, MS/MS 600.3 (100), 582.2 (8), 524.1 (7), 505.1 (3), 489.1 (11), 420.0 (6), 394.0 (36), 392.0 (20), 376.0 (3), 357.9 (3), 341.9 (1), 263.8 (16), [M-H+Cl]⁻ : *m/z* 654/656; [M-H+HCOOH]⁻ : *m/z* 664. [M+H]⁺ : *m/z* 620.3, MS/MS: *m/z* 602.2 (100), 584.3 (15.5), 574.3 (5.3), 489.2 (5.2), 359.9 (14.4), 341.9 (62.9), 260.9 (4.0), 232.9 (8.5). **620-c (Mr 619):** LC-ESI-MS (altern. +/-) from HPCCC fraction F36 (*Rt* 34.4 min), [M-H]⁻ : *m/z* 618.3, MS/MS 600.3 (5), 574.2 (3), 507.2 (25), 489.1 (100), 422.0 (32), 394.0 (84), 376.0 (35), 265.8 (32), [M-H+Cl]⁻ : *m/z* 654/656; [M-H+HCOOH]⁻ : *m/z* 664. [M+H]⁺ : *m/z* 620.3, MS/MS: *m/z* 602.2 (73), 507.1 (11.3), 359.9 (100), 342.9 (11.5), 222.8 (9.0). **620-d (Mr 619):** LC-ESI-MS (altern. +/-) from HPCCC fraction F54 (*Rt* 33.1 min), [M-H]⁻ : *m/z* 618.3, MS/MS 600.3 (90), 574.2 (4), 557 (7), 535.2 (45), 523.2 (35), 507.2 (85), 438.1 (60), 412.1 (90), 410.0 (100), 394.0 (25), [M-H+Cl]⁻ : *m/z* 654/656; [M-H+HCOOH]⁻ : *m/z* 664. [M+H]⁺ : *m/z* 620.3, MS/MS: *m/z* 602.3 (52.4), 507.1 (15.0), 465.1 (3.5), 343.9 (91.0), 326.9 (12.7), 206.9 (8.0). **620-e (Mr 619):** LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (*Rt* 33.8 min), [M-H]⁻ : *m/z* 618.3, MS/MS 600.3 (15), 574.2 (3), 535.2 (20), 523.2 (25), 507.2 (40), 482.2 (100), 438.1 (15), 412.1 (15), 410.0 (40), 394.0 (10), [M+H]⁺ : *m/z* 620.3, MS/MS: *m/z* 602.3 (39.6), 507.1 (9.4), 465.1 (1.7), 343.9 (100), 326.9 (14.9), 308.9 (4.1), 206.9 (9.2). **620-f (Mr 619):** LC-ESI-MS (altern. +/-) from HPCCC fraction F62 (*Rt* 35.4 min), [M-H]⁻ : *m/z* 618.3, MS/MS: *m/z* 600.3 (35), 574.3 (10), 535.2 (20), 523.2 (25), 507.2 (70), 482.1 (5), 438.1 (20), 412.0 (100), 410 (80), 394.0 (20), 376.0 (10); [M-H+Cl]⁻ : *m/z* 654/656; [M-H+HCOOH]⁻ : *m/z* 664. [M+H]⁺ : *m/z* 620.3, MS/MS: *m/z* 602.3 (35.7), 507.1 (6.7), 449.1 (7.8), 421.0 (1.2), 359.9 (100), 342.9 (3.0), 319.9 (10.0), 206.9 (4.6).

Opuntisine (metabolites 602-a – 602-c):

602-a (Mr 601): LC-ESI-MS (altern. +/-) from HPCCC fraction F33 (*Rt* 35.6 min), [M-H]⁻ : *m/z* 600.3, MS/MS: 582.2 (15), 489 (5), 420 (17), 394.0 (100), 377.9 (35), 265.8 (65). [M+H]⁺ :

m/z 602.3, MS/MS: m/z 584.2 (7.2), 566.2 (12.0), 557.3 (14.5), 491.1 (1.0), 455.1 (5.7), 396.0 (77.9), 350.9 (100), 284.9 (16.9).

602-b (tentative Mr 601): LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (R_t 36.2 min), $[M-H]^-$: no ionization. $[M+H]^+$: m/z 602.3, MS/MS: m/z 585.3 (18.6), 567.3 (42.6), 523.2 (5.1), 479.1 (15.0), 435.1 (9.1), 391.1 (32.8), 347.0 (12.6), 302.9 (35.4), 258.9 (50.3), 240.9 (7.8), 214.9 (100).

602-c (Mr 619): HPCCC F53-F58 (V_R 265-290 mL, K_D range: 3.05-4.65), LC-ESI-MS (altern. +/-) from HPCCC fraction F56 (R_t 33.4 min), $[M-H]^-$: m/z 618.3, MS/MS: m/z 482.2 (100), 273.9 (20), 255.9 (5), $[M-H+Cl]^-$: m/z 654/656, MS/MS: m/z 618.3 (10), 482.1 (100), 273.9 (10), $[M-H+HCOOH]^-$: m/z 664, MS/MS: m/z 618.3 (100), 482.1 (50), 273.8 (2). $[M+H]^+$: m/z 602, MS/MS: m/z 584.2 (52.5), 574.2 (6.4), 557.3 (4.3), 490.1 (4.0), 455.1 (7.7), 341.9 (100), 260.8 (9.3), 232.9 (19.2).

Opuntisine (metabolite 690-a, Mr 689):

HPCCC F20-F24 (V_R 100-120 mL, K_D range: 0.73 – 0.95), LC-ESI-MS MS (altern. +/-) from HPCCC fraction F22 (R_t 36.5 min), $[M-H]^-$: no ionization. $[M+H]^+$: m/z 690.4, MS/MS: see below; *off-line* ESI-MS (+) from HPCCC $[M+H]^+$: m/z 690, MS/MS: m/z 673.4, 655.4, 611.4, 549.1, 523.3, 479.2, 435.1, 411.0, 391.1, 347.0, 302.9, 258.9, 214.9.

Opuntisine (metabolite 690-b, Mr 689):

HPCCC F56-F64 (V_R 280-320 mL, K_D range: 3.82 – 14.0), LC-ESI-MS (altern. +/-) from HPCCC fraction F62 (R_t 33.6 min), $[M-H]^-$: m/z 688.4, MS/MS: m/z 602.3 (40), 575.2 (5), 421.0 (5), 507.2 (10), 359.9 (100). $[M+H]^+$ m/z 690.3, MS/MS: m/z 430.1 (10), 406.0 (100), 388.0 (10), 292.9 (10); *off-line* ESI-MS (+) from HPCCC $[M+H]^+$: m/z 648, MS/MS: m/z 527.12, 430.0, 405.9, 388.0, 292.8.

Opuntisine (metabolite 656, Mr 655):

HPCCC F52-F56 (V_R 260-280 mL, K_D range: 2.80 – 3.82), LC-ESI-MS (altern. +/-) from HPCCC fraction F53 (R_t 32.7 min), $[M-H]^-$: m/z 654.4, MS/MS: m/z 636.3 (15), 548.2 (5),

421.0 (10), 394.0 (100) 248.9 (10). $[M+H]^+$ m/z 656.4, MS/MS: m/z 493.1 (7), 406.0 (100), 388.0 (15), 258.9 (20); *off-line* ESI-MS (+) from HPCCC $[M+H]^+$: m/z 656, MS/MS: m/z 569.2, 493.2, 406.0, 387.9, 281.8, 258.9.

Supplement Table S1.

Calculation of chromatographic parameters of opuntisine A (1) and B (2) from the HPCCC experiment.

The metabolite elution times of HPCCC were converted over identified *elution/ retention volumes* V_R into their respective *partition ratio values* K_D (cf. equations 1 - 7). The V_R values for the elution of the respective cyclopeptides were determined with high accuracy by the *off-line* ESI-MS injection experiment (cf. Fig. 1). The calculation of *elution/retention volumes* V_R , *stationary phase retention* S_F , *partition ratio values* K_D is described by the following equations:

$$\text{Retention volume } V_R = \text{elution time [min]} \times \text{flow rate [mL/min]} \quad (1)$$

The value for S_F of the used solvent system was determined by equation (3) using V_C (125 mL), and V_M (37.63 mL) resulting in a S_F value of 69.9 %.

$$V_S = (V_C - V_M) \quad (2) \quad S_F = V_S / V_C \quad (3)$$

The S_F -value was re-calculated by use of the *extra column volume* V_{Ext} (7 mL) existing for the connecting periphery tubings in the HPCCC set-up, using equations (2 - 5)

$$\text{corrected } V_M = V_M - V_{ext} = 37.63 \text{ mL} - 7 \text{ mL} = 30.63 \text{ mL} \quad (4)$$

$$\text{Corrected } V_S = V_C - \text{corrected } V_M = 94.4 \text{ mL} \quad (5)$$

The *stationary phase retention factor* S_F was re-calculated as 75.5% (*corrected value*), and is the real percentage of stationary phase being present on the system during the HPCCC-experiment. A high S_F -value directly correlates to a higher resolution and efficiency in a CCC-separation.

$$\text{corrected } S_F = \text{corrected } V_S / V_C \quad (6)$$

For the *elution-mode*, the metabolite and solvent system specific *partition ratio* K_D values were calculated by the equation (7).

$$\text{corrected } K_D = (V_R - \text{corrected } V_M) / \text{corrected } V_S \quad (7)$$

From the begin of single solvent phase recovery in the *extrusion-mode* (cf. Fig. 2: F48, V_M = 240 mL), equation (8) will be applied:

$$\text{corrected } K_D = V_{CM} / (V_{CM} + V_C - V_R) \quad (8)$$

V_S : retained experimental stationary phase volume
 V_C : coil column volume/capacity
 V_M : volume of mobile phase take up to the coil at equilibrium of HPCCC
 S_F : stationary phase retention
 K_D : partition ratio
 V_{CM} : volume of switch volume to extrusion (end of classical mode elution)
 V_{ext} : external periphery volume

Corrected chromatographic CCC values V_M , V_S , K_D : re-calculation by use of external volume (V_{ext}) of periphery

No.	Selected ion traces compounds $m/z : [M+H]^+$	Prediction of partition ratio K_D by LC-ESI-MS $K_D = \text{Area (upper layer)} / \text{Area (lower layer)}$	HPCCC			$\Delta K_D = \text{Predicted } K_D - \text{HPCCC } K_D$
			<u>Peak range</u> Tube fractions Retention volume V_R ~ [mL]	K_D range K_D width	K_D Mean value	
1	m/z 620	1.32	F36 – F52 / 180 – 260	1.58 – 2.80	2.19	0.87
and 620 a – f		Derivatives 1a – 1f	only derivative 1b	1.22		
2	m/z 604	96	F64 - F66 / 320 - 330	14 – 42	28	68
				28		

Supplement Figure S1:

Predicted K_D -values of cyclopeptide alkaloids determined by LC-ESI-MS (pos. ionization), and calculated from integrated areas of selected ion traces in the HPCCC solvent system phase layers by equation ($K_D = A_{\text{upper}} / A_{\text{lower}}$) (Ito, 1996).

Predicted partition ratio $K_D = \text{Area selected single ion trace (upper layer)} / \text{Area selected single ion trace (lower layer)}$ $K_D = A_{\text{upper}} / A_{\text{lower}}$

Target cyclopetide alkaloids

LC-prediction

K_D (620) = 1.32

K_D (604) = 96

CCC-experiment

K_D (620) = 1.16 - 14

K_D (604) = > 14

Supplement Figures S2–a : ^1H NMR spectrum (700 MHz, methanol- d_4) of opuntisine A (**1**).

Supplement Figures S2-b : ^{13}C NMR spectrum (125 MHz, methanol- d_4) of opuntisine A (1).

Supplement Figures S2–c : COSY NMR spectrum (700 MHz, methanol-*d*₄) of opuntisine A (1).

Supplement Figures S2–d : TOCSY NMR spectrum (700 MHz, methanol-*d*₄) of opuntisine A (1).

Supplement Figures S2–e : ROESY NMR spectrum (700 MHz, methanol-*d*₄) of opuntisine A (1).

Supplement Figures S2–f : HSQC-DEPT NMR spectrum (700 MHz, methanol-*d*₄) of opuntisine A (**1**).

Supplement Figures S2–g : HMBC NMR spectrum (700 MHz, methanol-*d*₄) of opuntisine A (1).

Supplement Figures S3 – a : ^1H NMR spectrum (700 MHz, DMSO- d_6) of opuntisine B (2).

Supplement Figures S3 – b : ^{13}C NMR spectrum (125 MHz, DMSO- d_6) of opuntisine B (2).

Supplement Figures S3 – c : COSY NMR spectrum (700 MHz, DMSO-*d*₆) of opuntisine B (**2**).

Supplement Figures S3 – d : TOCSY NMR spectrum (700 MHz, DMSO-*d*₆) of opuntisine B (2).

Supplement Figures S3 – e :

ROESY NMR spectrum (700 MHz, DMSO-*d*₆) of opuntisine B (2).

Supplement Figures S3 – f : HSQC-DEPT NMR spectrum (700 MHz, DMSO-*d*₆) of opuntisine B (2).

Supplement Figures S3 – g : HMBC NMR spectrum (700 MHz, DMSO-*d*₆) of opuntisine B (**2**).

Supplement Figures S4 – a : TOCSY NMR spectrum (700 MHz, pyridine-*d*₅) of opuntisine A (1).

Supplement Figures S4 – b : TOCSY NMR spectrum (700 MHz, pyridine-*d*₅) of the (*S*)-MTPA ester of opuntisine A (1).

Supplement Figures S4 – c : TOCSY NMR spectrum (700 MHz, pyridine- d_5) of the (*R*)-MTPA ester of opuntisine A (1).

Supplement Figures S5: *Marfey* derivatization for determination of type and configuration of amino acids in **1** and **2**.

Detection of L-isoleucine; a) hydrolysate of **1** after derivatisation with FDAA; b) FDAA derivative of D-*allo*-isoleucine standard, c) FDAA derivative of D-isoleucine standard, d) FDAA derivative of L-*allo*-isoleucine standard, e) FDAA derivative of L-isoleucine standard.

Detection of L-glutamate; a) hydrolysate of **1** after derivatisation with FDAA; b) FDAA derivative of D -glutamate and c) L-glutamate standards, respectively.

Detection of L-phenylalanine; a) hydrolysate of **1** after derivatisation with FDAA; b) FDAA derivative of D-phenylalanine and c) L-phenylalanine standards, respectively.

Supplement Figures S5: *Marfey* derivatization for determination of type and configuration of amino acids in **1** and **2**.

Detection of L-isoleucine; a) hydrolysate of **2** after derivatisation with FDAA; b) FDAA derivative of D-*allo*-isoleucine standard, c) FDAA derivative of D-isoleucine standard, d) FDAA derivative of L-*allo*-isoleucine standard, e) FDAA derivative of L-isoleucine standard.

Detection of L-glutamate; a) hydrolysate of **2** after derivatisation with FDAA; b) FDAA derivative of D-glutamate and c) L-glutamate standards, respectively.

Detection of L-phenylalanin; a) hydrolysate of **2** after derivatisation with FDAA; b) FDAA derivative of D-phenylalanin and c) L-phenylalanin standards, respectively.

Supplement Figure S6: Fruit Analysis: Detection of Opuntisines in fruit tissues

a. HPLC-UV/VIS chromatogram (190-600 nm) of pulp extract; **b.** HPLC-extracted ion mass (620.3 Da) chromatogram of pulp extract; **c.** HPLC-ESI-extracted ion (604.3 Da) chromatogram of pulp extract; **d.** HPLC-UV/VIS chromatogram (190-600 nm) of seeds; **e.** HPLC-extracted ion mass (620.3 Da) chromatogram of seed extract; **f.** HPLC-extracted ion mass (604.3 Da) chromatogram of seed extract; **g.** HPLC-UV/VIS chromatogram (190-600 nm) of peel extract; **h.** HPLC-extracted ion mass (620.3 Da) chromatogram of peel extract; **i.** HPLC-extracted ion mass (604.3 Da) chromatogram of peel extract.