Electronic Supplementary Information

Intact NMR spectroscopy: slow high-resolution magic angle spinning chemical shift imaging

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Experimental Details

Sample preparation

The samples were packing into a standard 4-mm ZrO_2 rotor with an internal spacer at the bottom. A top Kel-F insert was used to secure the samples. No buffer or D_2O were added to avoid the displacement of metabolites during the sample spinning.

Food phantom: The phantom (Fig 1a) with fresh local food layers was prepared by punching the individual pre-sliced layer (ca. 1mm) with the open-edge rotor.

Wasp: Three individual wasps was caught locally – within few minutes apart – and immediately snap-freeze and stored under –80 °C until the acquisition. The frozen wasp was placed entirely inside the rotor with the head at the bottom. The body dimensions of the three wasps were nearly identical with 3 mm in diameter. This allowed a tight fix inside the rotor preventing body movement during the spinning. Among the three wasps, one was sacrificed for setting up the experiments: from field shimming, testing the phase cycling schemes (Fig S2) to setting up the spatial encoding parameters and the different TE (Fig S6). Other two wasps (WASP #1 and #2) were used to evaluate the HR-PASS CSI experiment and its data reproducibility (Fig S7). Spectra data of WASP #1 were discussed in the text (Fig 2). It should be noted that no visible destructions (or fluids) on the body were found after the HR-PASS CSI experiment.

NMR experiments

^{1}H experiments were performed on a Bruker Avance II spectrometer operating at 499.16 MHz, with a three channel Bruker 4-mm HR-MAS probe integrated with a homospoil z'-gradient. The gradient was operated by a 40 A amplifier. The fast- and slow-sample spinning were carried out with MAS II at rate of 4000 and 500 Hz, respectively, with ± less than 2 Hz. NMR spectra were collected with 16 k time domain points, 20 ppm sweep width, 1 s low-power presaturation pulse as a recycle delay, and with an echo time (TE = 2 × N number of rotor period τ_R) of 8 ms for the food phantom and 100 ms for the intact wasp. The number of accumulative scan was set to one-full phase cycling: 16 for HR-MAS CSI and 11 for HR-PASS CSI. In all CSI (including HR-PASS CSI) experiments, the gradient pulse length (δ) was set to a fixed value of 2 ms ($n \cdot \tau_R$); subsequently, the desired field-of-view (FOV) was adjusted by the gradient steps $t1_{CSI}$ together with the gradient amplitude $G_{Z'}$ (i.e. $FOV = [t1_{CSI}] \times [k \cdot {}^{1H}\gamma \cdot \delta \cdot G_{Z'}]^{-1}$, k is a scaling factor which is calibrated experimentally with a rigid cylinder). All CSI experiments were performed with a FOV of 8.3 mm attributed by 64 $t1_{CSI}$ and 10 % of $G_{Z'}$ for the phantom; or 32 $t1_{CSI}$ with 5 % for the wasp.

For the PASS sequence in HR-PASS CSI, 8-step $t1_{PASS}$ was carried out. Consequently, a total of 8 consecutive 2D HR-PASS CSI spectra with identical CSI parameters ($t1_{CSI}$, δ and $G_{Z'}$) were acquired, and each with a different set of interpulse delays for PASS. The delays can be found in Table 2 in [R.A. Wind et al., *Prog. Nucl. Magn. Reson. Spectrosc.,* 2006, **49**, 207.]

Data acquisitions and processing were carried out with the Bruker software package Topspin 3.2 and MATLAB.

For HR-PASS CSI, a summation of all 8 individual (i.e. 8-steps $t1_{PASS}$) 2D FID was carried by a Topspin command 'adsu'.

- The resultant summed F2 FID (i.e. chemical shift profile) was processed with zero filling and with an exponential apodization (with a LB = 0.5 Hz).
- The resultant summed F1 FID (i.e. spatial profile) was processed without 'zero' filling ($TD = t1_{CSI}$) and with a Gaussian apodization (LB = –1 Hz; GB = 0.5).
- A command 'xfb' in Topspin was applied to transform the summed 2D FID to the 2D spectrum with spatial profile in F1 and chemical shift in F2.

Each individual F2 slice projection spectrum was independently phased (except for those of wasp, which were phased with a magnitude mode), baseline correction 'abs', and referenced (to a doublet of alanine at 1.48 ppm). Peak assignments were assisted by Chenomx NMR suite 8.3, Human Metabolome Database Version 4.0 (www.hmbd.ca) and the corresponding references. Unfortunately, no supporting experiments (i.e. TOCSY, HSQC or J-RES) were performed to assist in the peak assignments.

Heatmap analysis

For each slice spectrum, R_{met} can be estimated by the individual metabolic integral (I_{met}): $R_{met} = [100\% \times I_{met}] / [N_{H\text{-}met} \times I_{spectral\ sum}]$, where $N_{H\text{-}met}$ is the number of corresponding protons, and $I_{spectral\ sum}$ is the total integral between 0.5-8.7 ppm excluding the water signal. This is followed by a colour coding scheme with red being the highest content and blue at zero. The peak integration of the individual metabolic signal (listed in Table S4) was carried out manually using MestreNova v8.1. It should be noted that the deduced R_{met} values should be considered as estimation because of the overlapping with the neighboring peaks.

Multivariate data analysis

The processed spectral data (by Topspin) were uploaded into MestreNova v8.1. The spectra (8.7 to – 0.5 ppm, excluding the water signal) were aligned. Each spectrum was divided into 0.02-ppm buckets. The signal area under each bucket was integrated and normalized to a constant sum of all integrals. The resultant data in the matrix (sample × chemical shift bucket) correspond to observation and variable, respectively, of the model study. The matrix was exported to SIMCA P13 to proceed with the multivariate modeling. It was scaled using the Pareto scaling. Unsupervised PCA (principal component analysis) was performed. The models were validated by assessing the values of R^2X, R^2Y, and Q^2.

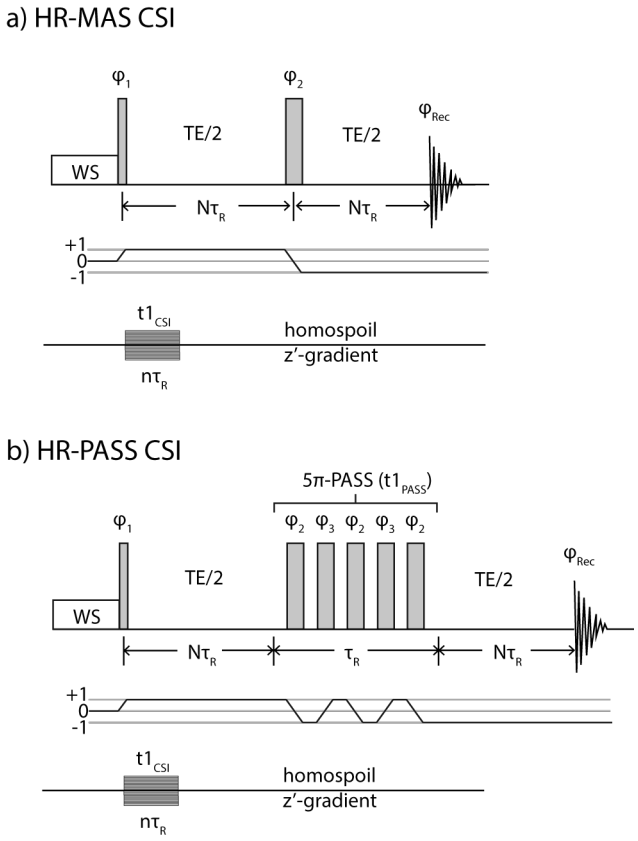

Fig S1. The NMR pulse sequence of (a) HR-MAS CSI and (b) HR-PASS CSI, along with the desired coherence transfer pathway which are determined by the phase cycling scheme: (a) φ_1 = ({0°}*4, {180°}*4, {90°}*4, {270°}*4); φ_2 = (0°, 90°, 180°, 270°); φ_R = (0°, 180°, 0°, 180°, 180°, 0°, 180°, 0°, 270°, 90°, 270°, 90°, 90°, 270°, 90°, 270°); and (b) 11-steps cogwheel scheme: φ_1 = 90°; φ_2 = (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) × 2π/11; φ_3 = 0° and φ_R = (0, 6, 1, 7, 2, 8, 3, 9, 4, 10, 5) × 2π/11. All interpulse delays are rotor synchronized (τ_R), including the echo delay (TE), the gradient pulse and the ensemble composite 5π in PASS. The PASS interpulse delays can be found in Table 2 in [R.A. Wind et al., *Prog. Nucl. Magn. Reson. Spectrosc.*, 2006, **49**, 207.] The Bruker Topspin pulse sequences (HR-MAS CSI and HR-PASS CSI) are available upon request [alan.wong@cea.fr].

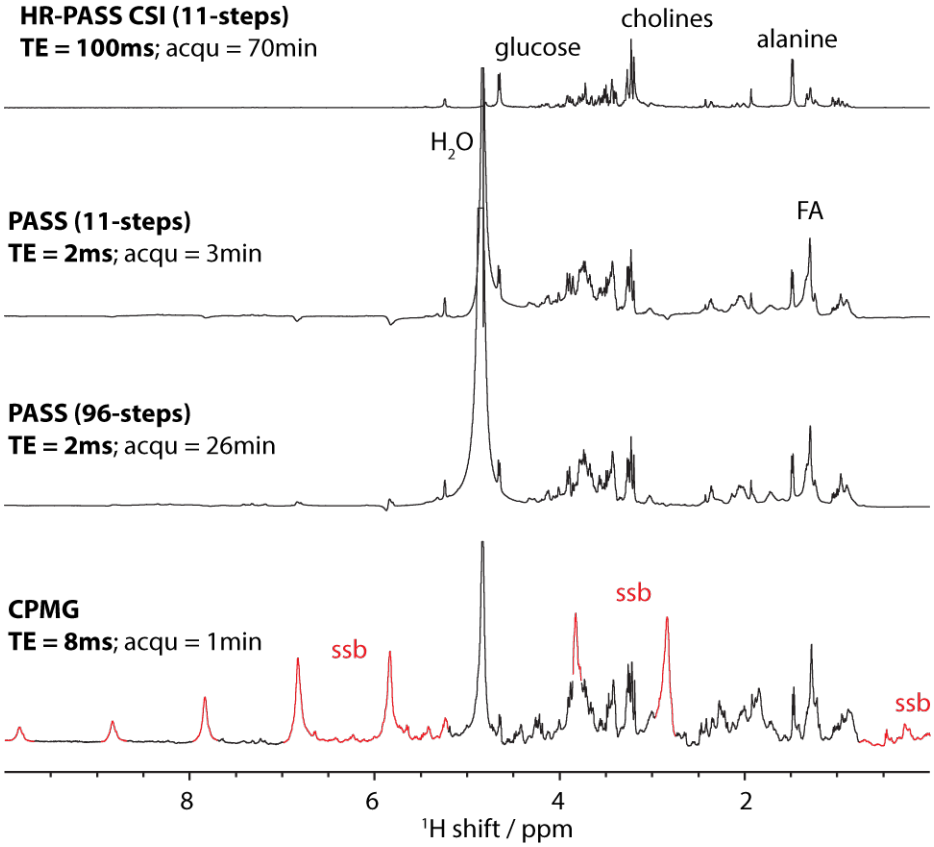

Fig S2 WASP. ^{1}H spectra (at 500 MHz) of an intact wasp (a sacrificed wasp used only for the experimental setup) spinning at 500 Hz. It illustrates the effect of the PASS experiment and the efficiency with the different phase-cycling scheme. Moreover, it also shows the impact of a long TE (100 ms) on the H_2O and fatty acids signals and their ssb.

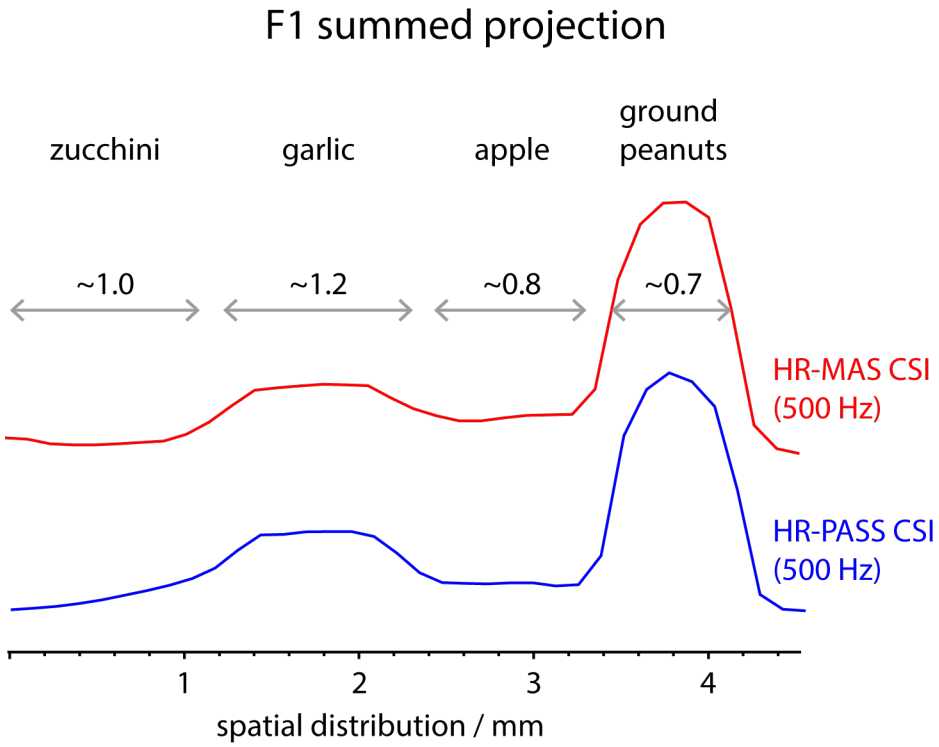

Fig S3 Phantom. The F1 summed projection (i.e. ^{1}H density spatial profile). It shows the distant spatial profile for the different food layers along z'.

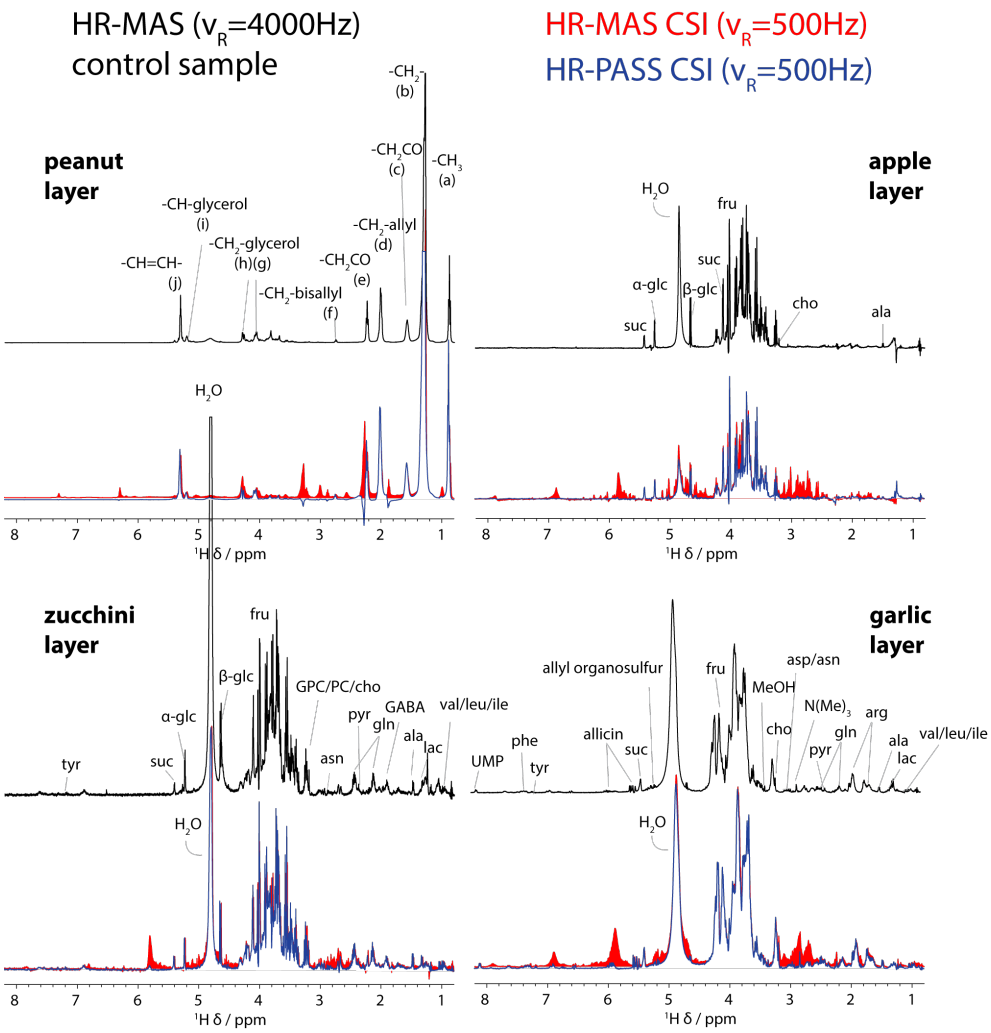

Fig S4 Phantom. ^{1}H spectral comparisons of the individual food layers. It demonstrates the ssb suppression on each layer with HR-PASS CSI. Moreover, the resultant HR-PASS CSI spectra (blue) correspond well with the HR-MAS NMR spectra (black) of the individual control samples. The tentative peak assignments are summarized in Table S1 and S2.

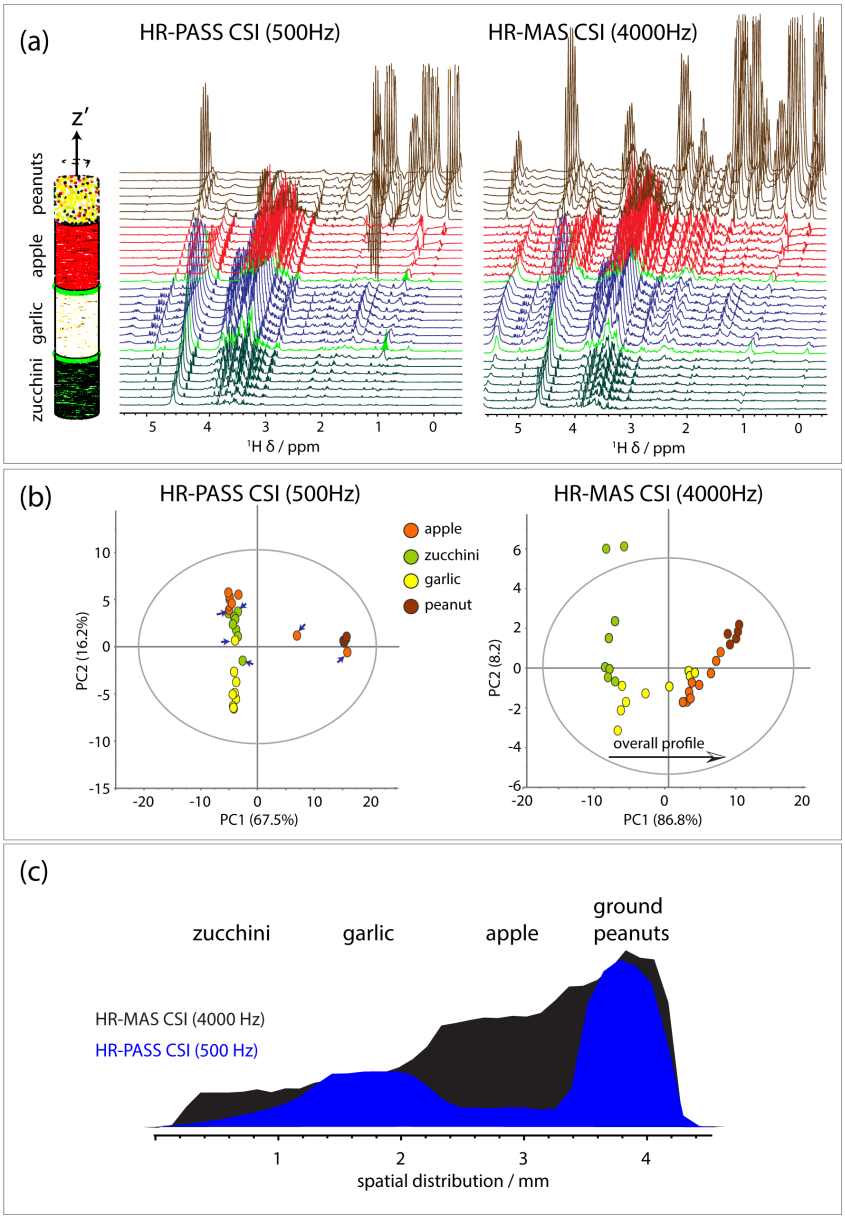

Fig S5 Phantom. Comparison between slow (500 Hz, top) and fast (4000 Hz, bottom) spinning: (a) Stacked plots of the slice spectra; and its (b) PCA with $R^2X = 0.976$, $Q^2 = 0.94$ for the slow spinning data, and with $R^2X = 0.993$, $Q^2 = 0.978$ for the fast spinning data; and (c) F1 summed projection (i.e. spatial profile).

The results (a,b) clearly show that the metabolic profiles are different between the slow and fast spinning. The blur arrows in the PCA of HR-PASS CSI (in b) indicate the interfaced spectral data between the layers. The black arrow in the PCA of HR-MAS CSI (in b) shows the directional changes in the individual spectral profile. (c) Shows the overall displacement of the metabolic fluids.

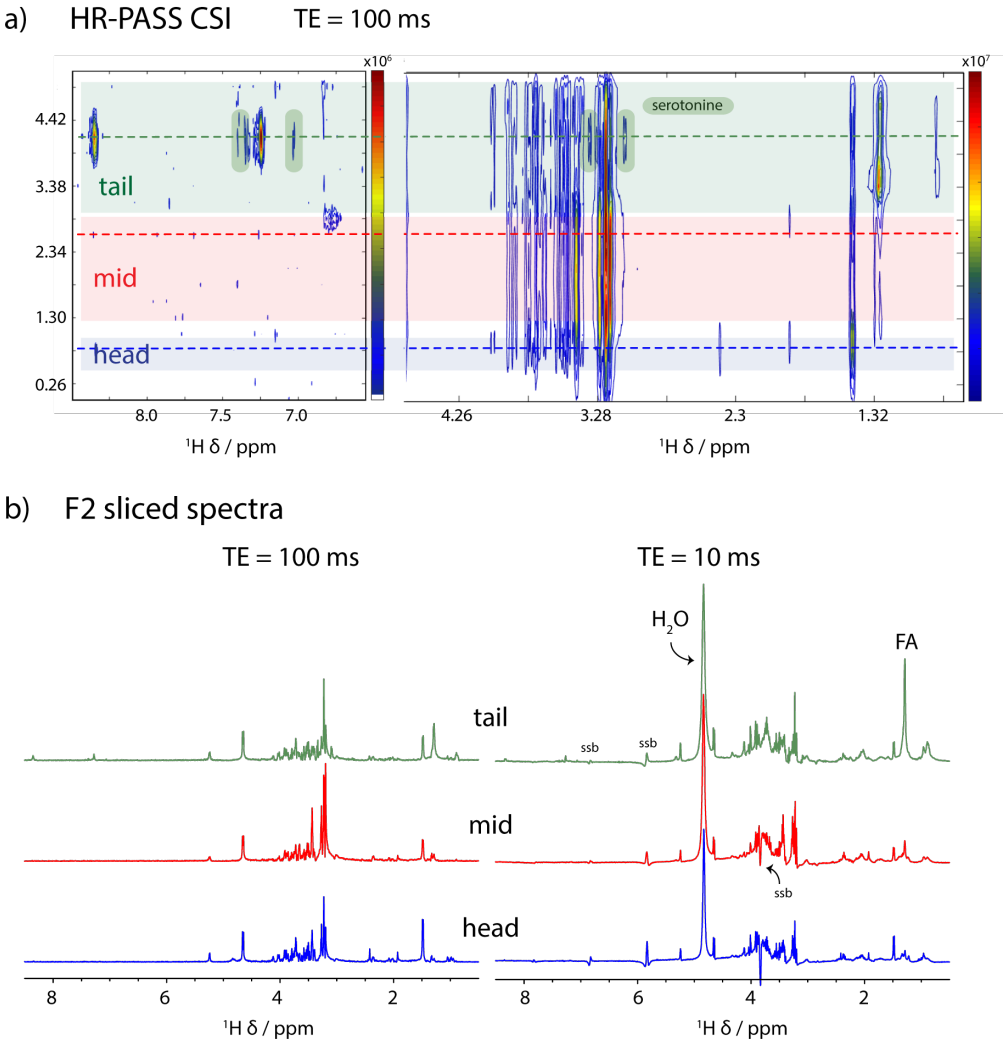

Fig S6 Wasp. (a) ^{1}H HR-PASS CSI. A 2D contour plot showing the different anatomical sections. A significant venom metabolite, serotonine, has been identified in the tail section. (b) Spectral comparison of the different TE on a scarified intact wasp (i.e. Fig S2). It shows the effect of long TE (with 100 ms) for suppressing both the isotopic and ssb of H_2O and fatty acids.

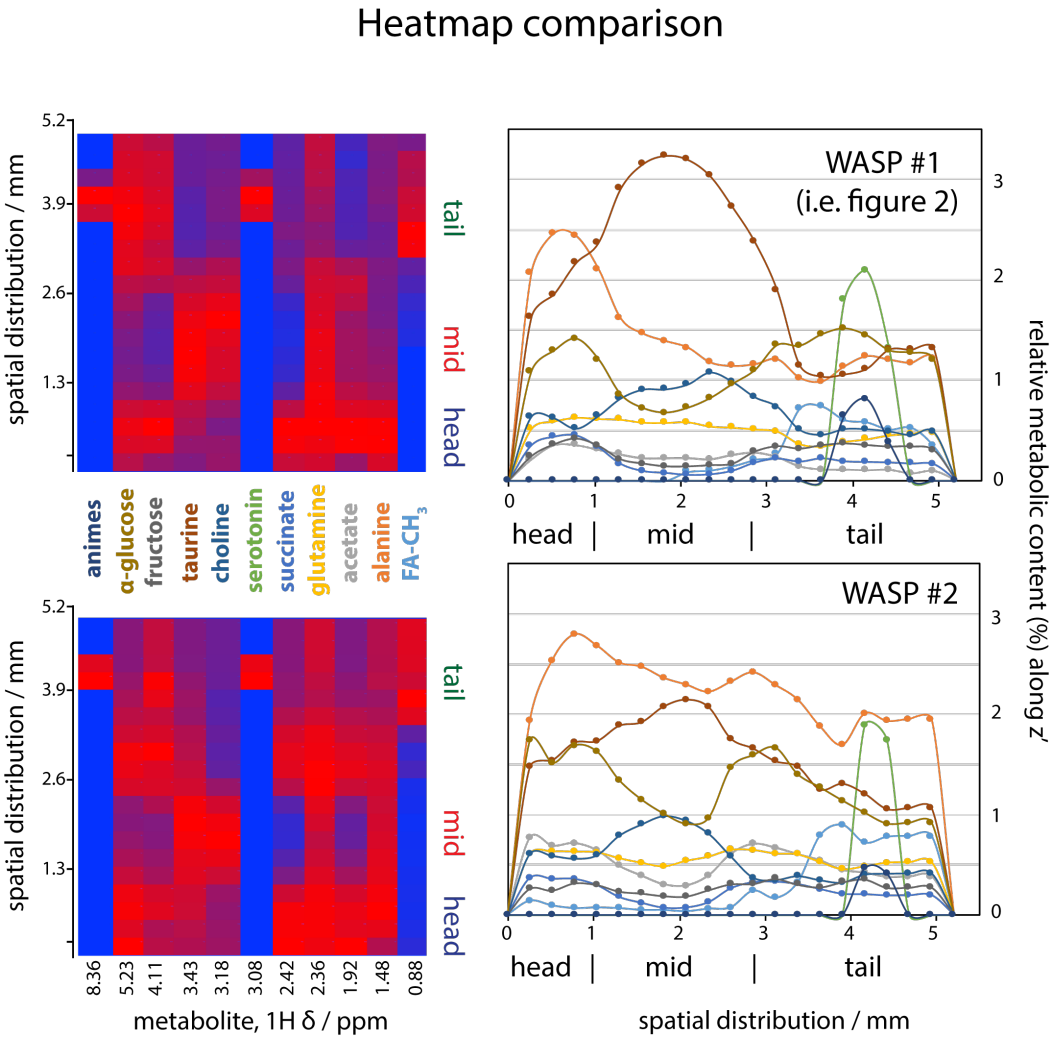

Fig S7 Wasp. Heatmap comparison between wasp #1 (top) vs #2 (bottom). The data of wasp #1 are discussed in the text (i.e. Fig 2). The color lines in the z' projection corresponds to those labelled in the heatmap. Note that the venom metabolites (i.e serotonin) are located in the tail section.

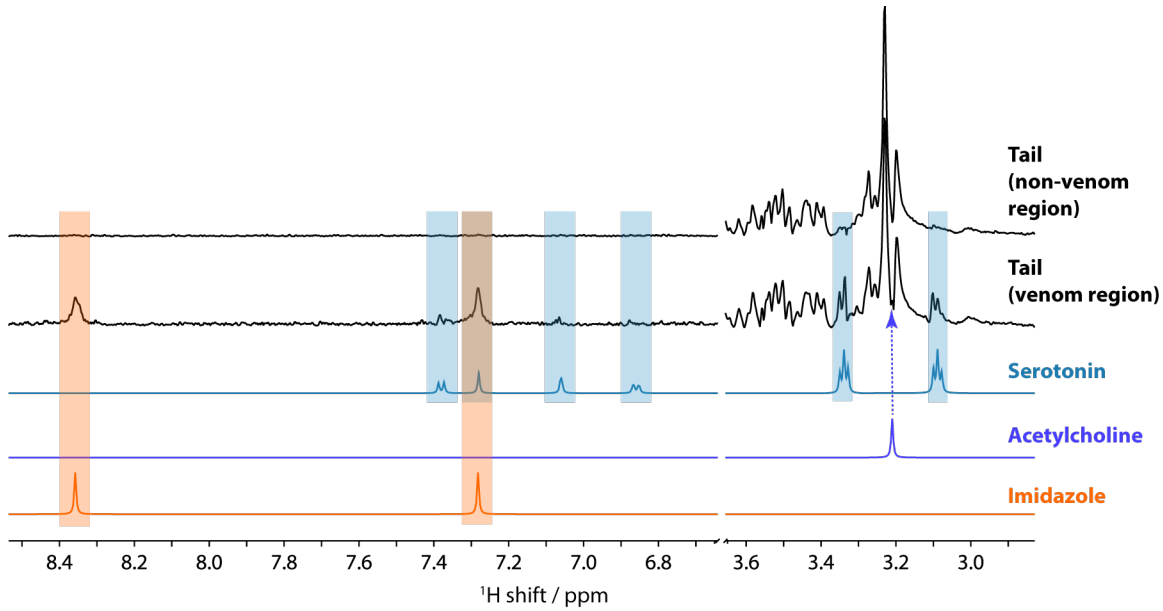

Fig S8 Wasp. Peak fitting, by Chenomx NMR, on the F2 slice spectra of the tail section in HR-PASS CSI. It shows the peak assignments of the two known venom metabolites (serotonin and acetylcholine), and also a 'possible' amine-derivatives (imidazole). Unfortunately, the study did not acquire supporting NMR spectra (such as TOCSY or HSQC or J-res) to confirm the assignments; however, the above fittings (especially for serotonin) offer a good level of confidence in the assignments.

Table S1: Tentative peak assignments from the individual HR-PASS CSI slice spectra of zucchini, garlic and apple

Peak [ppm] / (multiplicity)	Metabolite	Zucchini	Garlic	Apple
0.94 (t)	Leucine (leu)	x	x	x
0.96 (t)	Isoleucine (ile)	x	x	–
1.04 (d)	Valine (val)	x	x	–
1.18 (t)	unknown	x	x	x
1.33 (d)	Lactate (lac)	x	x	x
1.48 (d)	Alanine (ala)	x	x	x
1.91 (m)	Arginine (arg)	very low	x	–
2.3 (t)	GABA	x	–	x
2.14 (m), 2.45 (m)	Glutamine (gln)	x	?	–
2.38 (s)	Pyruvic	–	x	–
2.37 (dd), 2.67 (dd)	Malic	?	–	?
2.4 (s)	Succinate	?	–	–
2.85 (s)	N(Me)3	–	x	–
2.86 (dd), 2.96 (dd)	Asparagine (asn)	x	-	x
3.36 (s)	methonal	x	x	x
4.11 (m)	Fructose (fru)	x	x	x
4.64 (d), 5.2 (d)	Glucose (glc)	x	x	x
5.42 (d)	Sucrose	x	x	x
5.54 (m)	Allicin	–	x	–
6.53 (s)	Fumarate	x	x	–
6.9 (d), 7.2 (d)	Tyrosine (tyr)	x	x	–
7.3-7.4 (m)	Phenylalanine	x	x	–
8.11 (s)	UMP	–	x	–

Abbreviation: 'x' present; '?' questionable; '–' not present; (s) singlet; (d) doublet; (t) triplet; (m) multiple; GABA γ-aminobutyric acid; UMP Uridine monophosphate

Blue font: Peak used in the heatmap analysis

Reference: **[apple]** Martina Vermathen, Mattia Marzorati, Gaëlle Diserens, Daniel Baumgartner, Claudia Good, Franz Gasser, Peter Vermathen, Metabolic profiling of apples from different production systems before and after controlled atmosphere (CA) storage studied by ^{1}H high resolution-magic angle spinning (HR-MAS) NMR, *Food Chemistry* **2017**, *233*, 391–400; **[garlic]** (a) Tingfu Liang, Feifei Wei, Yi Lu, Yoshinori Kodani, Mitsuhiko Nakada, Takuya Miyakawa, and Masaru Tanokura, Comprehensive NMR Analysis of Compositional Changes of Black Garlic during Thermal Processing, *J. Agric. Food Chem.* **2015**, *63*, 683–691; (b) Covadonga Lucas-Torres, Gaspard Huber, Atsuyuki Ichikawa, Yusuke Nishiyama, Alan Wong, HR-μMAS NMR-Based Metabolomics: Localized Metabolic Profiling of a Garlic Clove with μg Tissues, *Anal. Chem.* **2018**, *90*, 13736–13743. **[zucchini]** Ana Cristina Abreu, Luis Manuel Aguilera-Saéz, Araceli Peña, Mar García-Valverde, Patricia Marín, Diego L. Valera, Ignacio Fernańdez, NMR-Based Metabolomics Approach To Study the Influence of Different Conditions of Water Irrigation and Greenhouse Ventilation on Zucchini Crops, *J. Agric. Food Chem.* **2018**, *66*, 8422–8432.

Table S2: Tentative peak assignments from the individual HR-PASS CSI slice spectra of peanut

Peak [ppm]	FA-peak
0.88	-CH3
1.28	-CH2-
1.58	-CH2bCO
2.02	-CH2-allyl
2.25	-CH2aCO
2.75	-CH2-bisallyl
3.7	?
3.83	?
4.07	-CH2-glycerol
4.28	-CH2-glycerol
5.21	-CH-glycerol
5.32	-CH = CH-

Abbreviation: '?' questionable

Blue font: Peak used in the heatmap analysis.
The shift range are summarized in Tab S4.

Table S3: Tentative peak assignments from the individual HR-PASS CSI slice spectra of head, mid and tail section (excluding the fatty acid peaks)

Metabolite	**peak (ppm)**	**Head**	**Mid**	**Tail**
Leucine (leu)	0.96	x	–	–
Isoleucine (ile)	1.00	x	–	–
Valine (val)	1.02	x	–	x
Lactate (lac)	1.33	x	x	x
Alanine (ala)	1.48	x	x	x
Acetate (ace)	1.92	x	x	–
Glutamate	2.03, 2.35	x	x	x
Succinate (suc)	2.42	x	–	x
Serotonin*	3.08, 3.35, 6.86, 7.06, 7.27, 7.37	–	–	x
Choline (cho)	3.19	x	x	x
Acetylcholine (ACh)	3.20, 3.73	–	–	x
Phosphocholine (PC)	3.20	x	x	–
Glycerophosphocholine (GPC)	3.22	x	x	x
Taurine (tau)	3.26, 3.43	x	x	–
β-glucose (β-glc)	4.65	x	x	x
α-glucose (α-glc) / or trehalose	5.24	x	x	x
Fructose	4.11, 4.04, 4.01	x	–	x
Venom active animes (imidazole ?)*	7.27, 8.34	–	–	x

Abbreviation: ‘x’ present; ‘?’ questionable; ‘–‘ not present;

* according to Chenomx Profiler assignment (Fig S9)

Blue font: Peak used in the heatmap analysis. The shift range are summarized in Table S4.

Reference: [1] Moreno M, Giralt, E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: Melittin, apamin and mastoparan. *Toxins (Basel)*. **2015**, *7*, 1126–1150; [2] Kapranas A, Snart CJP, Williams H, Hardy ICW, Barrett DA. Metabolomics of aging assessed in individual parasitoid wasps. *Sci Rep*. **2016**, *6*, 34848.

Table S4: The chemical shift range used for the signal integration I_{met} in the heatmap.

Metabolite	**Range [ppm] (multiplicity)**	**Metabolite**	**Range [ppm] (multiplicity)**
Food phantom		**WASP #1**	
Tyrosine	7.23 - 7.16 (d)	Anime?	8.40 - 8.32 (s)
Fumarate	6.33 - 6.52 (s)	Glucose	5.26 - 5.21 (d)
Allicin	5.60 - 5.47 (4s)	Fructose	4.14 - 4.10 (br)
Sucrose	5.45 - 5.38 (d)	Taurine	3.45 - 3.41 (t)
UFA	5.37 - 5.25 (s)	Choline	3.21 - 3.16 (s)
Glyercol	5.24 - 5.17 (s)	Serotonin	3.11 - 3.05 (t)
Glucose	4.67 - 4.61 (d)	Succinate	2.44 - 2.40 (s)
Fructose	4.13 - 4.1 (br)	Glutamine	2.39 - 2.32 (m)
Methanol	3.37 - 3.35 (s)	Acetate	1.94 - 1.90 (s)
Choline	3.20 - 3. 17 (s)	Alanine	1.50 - 1.45 (d)
$N(Me)_3$	2.86 - 2.83 (s)	FA-CH_3	0.92 - 0.85 (br)
Pyruvate	2.39 - 2.37 (s)		
Glutamine	2.20 - 2.10 (m)		
Arginine	1.96 - 1.86 (m)		
Alanine	1.50 - 1.46 (d)		
FA-CH_2	1.32 - 1.24 (br)		
Valine	1.06 - 1.02 (d)		
FA-CH_3	0.91 - 0.86 (br)		

Abbreviation: (s) singlet; (d) doublet; (t) triplet; (m) multiple; (br) broad