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Molecular characterisation of an atypical coconut-like odour in cocoa

Electronic Supplementary Information

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GC–O/FID system

A Trace Gas Chromatograph Ultra (Thermo Fisher Scientific, Dreieich, Germany) was equipped with a cold on-column injector, a flame ionization detector (FID), and a sniffing port. The sniffing port was custom-made as detailed in Steinhaus et al. (2008) J Agric Food Chem 56:4120–4127. The fused silica column was either a DB-FFAP, a DB-5, both 30 m × 0.32 mm i.d., 0.25 µm film (Agilent, Waldbronn, Germany), or a chiral MEGA-DEX DAC-Beta, 30 m × 0.25 mm i.d., 0.15 µm film (BGB Analytik, Rheinfelden, Germany), which was based on a diacetyl-*tert*-butylsilyl-modified β-cyclodextrin in phenylmethylpolysiloxane. The carrier gas was helium at a constant flow of 2 mL/min. The injection volume was 1 µL. The oven temperature was 40 °C for 2 min and then was increased by 6 °C/min to 230 °C (DB-FFAP), by 6 °C/min to 240 °C (DB-5), or by 2 °C/min to 220 °C (MEGA-DEX DAC-Beta). Final temperatures were held for 5 min. The column effluent was divided 1:1 using a deactivated Y-shaped glass splitter (CHM, Fridolfing, Germany) and two deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) directing the effluent to the FID (base temperature 250 °C) and the sniffing port (base temperature 230 °C), respectively.

During a GC–O/FID run, the assessor placed the nose closely above the sniffing port and evaluated the odour of the effluent. Whenever an odour was perceived, the retention time and the odour quality were noted in the FID chromatogram plotted by a recorder. A linear retention index (RI) was calculated for each odour-active compound from its retention time and the retention times of adjacent *n*-alkanes by linear interpolation as detailed in van den Dool and Kratz (1963) J Chromatogr A 11:463–471.

GC–HRMS system

A Trace 1310 gas chromatograph (Thermo Fisher Scientific) was equipped with a TRI Plus RSH autosampler and a programmed temperature vaporizing (PTV) injector. The fused silica column used was a either a DB-FFAP or a DB-5, both 30 m × 0.25 mm i.d., 0.25 µm film (Agilent). The carrier gas was helium at a constant flow of 1 mL/min. The injection volume was 1 µL. The oven temperature was 40 °C for 2 min and then was increased by 6 °C/min to 230 °C (DB-FFAP) or 240 °C (DB-5). The final temperature was held for 5 min. The column end was connected to a Q Exactive GC Orbitrap mass spectrometer (Thermo Fisher Scientific) operated in high resolution mode. Mass spectra in the electron ionization (EI) mode were obtained at 70 eV and a scan range of *m*/*z* 35−350. Mass spectra in the chemical ionization (CI) mode were obtained at 70 eV using isobutane as reagent gas and a scan range of *m*/*z* 85−350. Data evaluation was accomplished by using the Xcalibur software (Thermo Fisher Scientific).

Heart-cut GC–GC–HRMS system

A Trace 1310 gas chromatograph (Thermo Fisher Scientific) was equipped with a TRI Plus RSH autosampler, a programmed temperature vaporizing (PTV) injector, an FID detector, and a custom-made sniffing port. The fused silica column used was a DB-FFAP, 30 m × 0.25 mm i.d., 0.25 µm film (Agilent). The carrier gas was helium at a constant flow of 1 mL/min. The injection volume was 1 µL. The oven temperature was 40 °C for 2 min and then was increased by 6 °C/min to 160 °C and finally by 40 °C/min to 230 °C. The final temperature was held for 5 min. A Dean's switch (Thermo Fisher Scientfic) was employed to toggle the effluent of the column between the FID and the sniffing port on the one

hand and a cold trap cooled by liquid nitrogen on the other hand. The cold trap was connected to another Trace 1310 gas chromatograph equipped with a fused silica column DB-1701, 30 m × 0.25 mm i.d, 0.25 µm film (Macherey-Nagel, Düren, Germany) or a fused silica column MEGA-DEX DAC-Beta, 30 m × 0.25 mm i.d., 0.15 µm film (BGB Analytik). When using the DB-1701 column, the oven temperature was 40 °C for 2 min and then was increased by 6 °C/min to 240 °C. When using the MEGA-DEX DAC-Beta column, the initial oven temperature was 40 °C for 2 min and then was increased by 2 °C/min to 220 °C. Final temperatures were held for 5 min. The end of the column in the second gas chromatograph was coupled to a Q Exactive GC Orbitrap mass spectrometer (Thermo Fisher Scientific) operated in high resolution mode. Mass spectra in the chemical ionization (CI) mode were obtained at 70 eV using isobutane as reagent gas and a scan range of *m*/*z* 85−350. Data evaluation was accomplished by using the Xcalibur software (Thermo Fisher Scientific).

Before the analysis of cocoa volatile isolates for odorant quantitation, the retention times of the target compounds and the internal standards in the first and second dimensions were determined by analysis of reference mixtures. During the subsequent analysis of cocoa volatile isolates, a heart-cut of the effluent of the first column containing the respective target compound and its internal standard was transferred to the cold trap. Analysis of the refocussed substances in the second dimension was started by turning off the cooling of the trap and starting the temperature programme of the second oven as well as the mass spectrometer.

Table S1 Internal standards, quantifier ions, and calibration lines used in the quantitation assays

Odorant	Standard	Quantifier ions (m/z)		Calibration line equation[a]	R^2
		Analyte	Standard		
34	($^2H_2$)-**34**	143	145	y = 0.7864x + 0.0709	0.999
36	($^2H_2$)-**36**	157	159	y = 0.6365x + 0.1173	0.993
37	($^2H_2$)-**34**	141	145	y = 0.7688x − 0.0584	0.999
42	($^2H_2$)-**42**	171	173	y = 0.7061x + 0.0389	0.996
44	($^2H_{2-4}$)-**44**	171	173–175	y = 1.0049x − 0.0946	0.998
45	($^2H_{2-4}$)-**44**	169	173–175	y = 0.7629x − 0.0726	0.999

[a]y = peak area counts standard / peak area counts analyte; x = concentration standard (µg/mL) / concentration analyte (µg/mL)

Table S2 Concentrations of lactones in three samples of fermented cocoa with an atypically pronounced coconut odour (C1, C2, C3) and in the reference cocoa sample (REF)

Sample	Odorant	Concentration (µg/kg)			
		Exp. 1[a]	Exp. 2[a]	Exp. 3[a]	Mean[b] ± SD[c] (%)
C1	**34**	0.209	0.180	0.196	0.195 ± 0.015 (8%)
	36	155	138	163	152 ± 12 (8%)
	37	0.291	0.331	0.336	0.319 ± 0.024 (8%)
	42	49.3	51.4	53.1	51.3 ± 1.9 (4%)
	44	67.1	70.4	90.6	76.0 ± 12.7 (17%)
	45	1570	1590		1580 ± 14 (1%)
C2	**34**	0.815	0.731	0.686	0.744 ± 0.065 (9%)
	36	191	190	192	191 ± 1 (1%)
	37	1.88	1.34	1.55	1.59 ± 0.27 (17%)
	42	472	354		413 ± 84 (20%)
	44	529	392		461 ± 97 (21%)
	45	625	532	553	570 ± 49 (9%)
C3	**34**	0.379	0.351	0.336	0.355 ± 0.022 (6%)
	36	164	176	146	162 ± 15 (9%)
	37	0.959	0.889	0.852	0.900 ± 0.055 (6%)
	42	49.4	53.6	53.9	52.3 ± 2.5 (5%)
	44	90.3	97.4	89.3	92.3 ± 4.4 (5%)
	45	224	198	210	210 ± 8 (4%)
REF	**34**	0.410	0.438	0.418	0.422 ± 0.015 (3%)
	36	126	98.9	150	125 ± 26 (21%)
	37	0.682	0.722	0.713	0.706 ± 0.017 (2%)
	42	110	108		109 ± 2 (1%)
	44	7.57	6.39		6.98 ± 0.59 (8%)
	45	81.2	76.8	100	86.1 ± 12.5 (15%)

[a]Individual concentrations were rounded to three significant digits

[b]Mean values were calculated from the exact concentrations of the individual experiments and finally rounded to three significant digits

[c]Standard deviation

Table S3 Olfactory profiles of the reference cocoa sample spiked with δ-2-decenolactone, the reference sample without addition, and the sample C1 with the atypically pronounced coconut note.

Odour	Reference cocoa spiked with δ-2-decenolactone		Reference cocoa		Cocoa C1 with pronounced coconut note	
	Mean[a]	SD[b]	Mean[a]	SD[b]	Mean[a]	SD[b]
Coconut	2.00	0.91	0.81	0.56	2.08	0.94
Vanilla	1.01	0.65	1.25	0.78	1.38	0.84
Honey	1.49	0.80	1.59	0.87	1.13	0.70
Banana	1.14	0.84	0.99	0.73	1.05	0.80
Fruity	1.15	0.80	0.93	0.58	1.19	0.70
Vinegar	1.73	0.85	1.95	0.78	1.39	0.74
Earthy	1.07	0.74	0.97	0.70	0.86	0.69
Malty	1.41	0.70	1.41	0.96	1.32	0.88

[a]Mean values of the intensity ratings of 19 trained assessors; intensities were rated on a scale from 0 to 3 with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

[b]Standard deviation