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Impact of processing on important cocoa off-flavour compounds

Electronic Supplementary Information

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Heart-cut GC–GC–MS system

A Trace GC Ultra (Thermo Fisher Scientific, Dreieich, Germany) was equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a cold on-column injector. The column was either a DB-FFAP or a DB-5, both 30 m × 0.32 mm i.d., 0.25 µm film thickness (Agilent Technologies, Waldbronn, Germany). The carrier gas was helium at a constant pressure of 115 kPa. The injection volume was 2 µL. The oven start temperature was 40 °C (2 min), the subsequent temperature gradients ranged from 6 to 40 °C/min, and the final temperatures were 230−240 °C (5 min). The end of the column was connected to a moving column stream switching (MCSS) system (Thermo Fisher Scientific). The make-up gas was helium at 50 kPa. The MCSS system transferred the column effluent time-programmed via deactivated fused silica capillaries (0.32 mm i.d.) either simultaneously to an FID (250 °C base temperature) and a custom-made sniffing port (230 °C base temperature) or to a liquid nitrogen-cooled trap inside the oven of a second gas chromatograph (250 °C transfer line temperature). The second GC was a CP 3800 (Varian, Darmstadt, Germany) equipped with a liquid nitrogen-cooled cold trap for the refocusing of the heart-cut. The GC column in the second oven was either a DB-1701 or a DB-FFAP, both 30 m × 0.25 mm i.d., 0.25 µm film thickness (Agilent Technologies). The oven start temperature was 40 °C (2 min), the subsequent temperature gradients ranged from 6 to 30 °C/min, and the final temperatures were 230−240 °C (5 min). The end of the second column was connected to a Saturn 2200 ion trap mass spectrometer (Varian) operated in the CI mode with methanol as the reagent gas and a scan range of *m*/*z* 60−250. Data were evaluated using the MS Workstation software (Varian).

Heart-cut GC–GC–HRMS system

A Trace GC 1310 (Thermo Fisher Scientific) was equipped with a TriPlus RSH autosampler and a PTV injector. The column was a DB-FFAP, 30 m × 0.32 mm i.d., 0.25 µm film thickness (Agilent Technologies). The carrier gas was helium at a constant flow of 1 mL/min. The injection volume was 1 µL. The oven temperature programme was 40 °C (2 min), 6 °C/min to 230 °C (5 min). The end of the column was connected to a Deans Switch (Thermo Fisher Scientific) which transferred the column effluent time-programmed via deactivated fused silica capillaries (0.1 mm i.d.) either simultaneously to an FID (250 °C base temperature) and a custom-made sniffing port (230 °C base temperature) or to a liquid nitrogen-cooled cold trap. The cold trap was connected to a second Trace GC 1310 (Thermo Fisher Scientific) which was also equipped with an FID (250 °C base temperature) and a custom-made sniffing port (230 °C base temperature). The GC column in the second oven was either a DB-1701 (Macherey-Nagel, Düren, Germany) or a BGB-176 (BGB Analytik, Rheinfelden, Germany), both 30 m × 0.25 mm i.d., 0.25 µm film thickness. The oven temperature programme was 40 °C (2 min), 6 °C/min to 240 °C (5 min) for the DB-1701 column and 40 °C (2 min), 2 °C/min to 200 °C (5 min) for the BGB-176 column. The end of the second GC column was connected to a Q Exactive GC Orbitrap mass spectrometer (Thermo Fisher Scientific) operated in the CI and high-resolution mode with isobutane as the reagent gas and a scan range of *m*/*z* 85−350. Data were evaluated using the Xcalibur software (Thermo Fisher Scientific).

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		
2-Methoxyphenol	($^2H_3$)-2-Methoxyphenol	125	128	y = 1.0524x − 0.0401	1.000
3-Methylphenol	($^2H_{4\text{-}8}$)-4-Methylphenol	109	113–117	y = 1.2545x − 0.1768	0.999
4-Methylphenol	($^2H_{4\text{-}8}$)-4-Methylphenol	109	113–117	y = 1.2171x − 0.1239	0.998
3-Ethylphenol	($^2H_2$)-4-Ethylphenol	123	125+126	y = 0.9717x − 0.1067	0.999
4-Ethylphenol	($^2H_2$)-4-Ethylphenol	123	125+126	y = 0.9156x − 0.0334	1.000
3-Propylphenol	($^2H_2$)-4-Propylphenol	137	139	y = 0.8121x − 0.1575	0.999
(−)-Geosmin	($^2H_3$)Geosmin	165	168	y = 0.1822x – 0.0809	0.998
3-Methyl-1*H*-indole	($^2H_{7\text{-}8}$)-3-Methyl-1*H*-indole	132	139+140	y = 0.8152x + 0.0798	0.999
1*H*-Indole	($^2H_6$)-1*H*-Indole	118	123+124	y = 0.8685x + 0.0319	0.998
MDMF	($^2H_3$)MDMF	143	146	y = 1.2365x + 0.0765	1.000

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

Table S2 Changes in the concentrations of crucial off-flavour compounds in sample SOF1 caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	2-Methoxyphenol	877	905	831	871 ± 37 (4%)
	3-Methylphenol	559	606	560	575 ± 27 (5%)
	4-Methylphenol	463	504	460	476 ± 25 (5%)
	3-Ethylphenol	191	178	167	179 ± 12 (7%)
	4-Ethylphenol	315	338	308	320 ± 16 (5%)
	3-Propylphenol	27.0	29.2	29.1	28.4 ± 1.2 (4%)
125 °C	2-Methoxyphenol	864	861	934	886 ± 41 (5%)
	3-Methylphenol	500	519	532	517 ± 16 (3%)
	4-Methylphenol	439	446	465	450 ± 13 (3%)
	3-Ethylphenol	162	153	160	158 ± 5 (3%)
	4-Ethylphenol	297	318	308	308 ± 11 (3%)
	3-Propylphenol	22.7	24.9	18.2	21.9 ± 3.4 (16%)
140 °C	2-Methoxyphenol	1040	1080	1030	1050 ± 26 (3%)
	3-Methylphenol	602	625	607	611 ± 12 (2%)
	4-Methylphenol	505	531	522	519 ± 13 (3%)
	3-Ethylphenol	188	194	191	191 ± 3 (2%)
	4-Ethylphenol	378	361	365	368 ± 9 (2%)
	3-Propylphenol	24.4	27.8	32.0	28.1 ± 3.8 (14%)

Table S3 Changes in the concentrations of crucial off-flavour compounds in sample SOF2 caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	2-Methoxyphenol	373	406	399	393 ± 17 (4%)
	3-Methylphenol	311	306	320	312 ± 7 (2%)
	4-Methylphenol	156	160	155	157 ± 3 (2%)
	3-Ethylphenol	90.2	110	90.2	96.7 ± 11.3 (12%)
	4-Ethylphenol	273	210	224	236 ± 33 (14%)
	3-Propylphenol	15.1	14.8	12.9	14.3 ± 1.2 (8%)
125 °C	2-Methoxyphenol	478	482	504	488 ± 14 (3%)
	3-Methylphenol	323	325	340	329 ± 9 (3%)
	4-Methylphenol	175	174	180	176 ± 3 (2%)
	3-Ethylphenol	83.7	83.5	72.3	79.8 ± 6.5 (8%)
	4-Ethylphenol	206	212	209	209 ± 3 (1%)
	3-Propylphenol	7.55	6.31		6.93 ± 0.88 (13%)
140 °C	2-Methoxyphenol	458	450	428	445 ± 16 (3%)
	3-Methylphenol	298	299	308	302 ± 6 (2%)
	4-Methylphenol	164	170	175	170 ± 6 (3%)
	3-Ethylphenol	87.0	81.0	84.5	84.2 ± 3.0 (4%)
	4-Ethylphenol	251	246	250	249 ± 3 (1%)
	3-Propylphenol	5.26	6.73	4.68	5.56 ± 1.06 (19%)

Table S4 Changes in the concentrations of crucial off-flavour compounds in the reference sample caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	2-Methoxyphenol	57.1	59.6	56.5	57.7 ± 1.6 (3%)
	3-Methylphenol	5.22	4.91	4.78	4.97 ± 0.23 (5%)
	4-Methylphenol	16.4	16.3	15.4	16.0 ± 0.6 (3%)
	3-Ethylphenol	2.28	2.05		2.17 ± 0.16 (8%)
	4-Ethylphenol	33.4	40.4	31.6	35.1 ± 4.6 (13%)
	3-Propylphenol	0.289	0.239		0.264 ± 0.035 (13%)
125 °C	2-Methoxyphenol	72.1	64.7	65.8	67.5 ± 4.0 (6%)
	3-Methylphenol	4.24	4.58	4.36	4.39 ± 0.17 (4%)
	4-Methylphenol	15.6	17.1	17.0	16.6 ± 0.8 (5%)
	3-Ethylphenol	5.84	7.54	4.57	5.98 ± 1.49 (25%)
	4-Ethylphenol	43.9	47.3	45.9	45.7 ± 1.7 (4%)
	3-Propylphenol	0.400	0.294		0.347 ± 0.075 (22%)
140 °C	2-Methoxyphenol	62.3	63.1	72.3	65.9 ± 5.6 (8%)
	3-Methylphenol	7.07	6.12	6.17	6.45 ± 0.53 (8%)
	4-Methylphenol	18.1	23.3	23.1	21.5 ± 2.9 (14%)
	3-Ethylphenol	3.13	3.28		3.21 ± 0.11 (3%)
	4-Ethylphenol	66.2	61.4	65.8	64.5 ± 2.7 (4%)
	3-Propylphenol	0.277	0.273	0.325	0.292 ± 0.029 (10%)

Table S5 Changes in the concentrations of crucial off-flavour compounds in sample MOF1 caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	(−)-Geosmin	6.32	5.87	6.60	6.26 ± 0.37 (6%)
	3-Methyl-1*H*-indole	2.08	2.79	2.30	2.39 ± 0.36 (15%)
	1*H*-Indole	26.2	24.5	23.5	24.7 ± 1.4 (6%)
	MDMF	27.7	27.2	24.3	26.4 ± 1.8 (7%)
125 °C	(−)-Geosmin	6.30	6.66	5.60	6.19 ± 0.54 (9%)
	3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
	1*H*-Indole	60.5	69.5	72.4	67.5 ± 6.2 (9%)
	MDMF	37.5	38.6	38.5	38.2 ± 0.6 (2%)
140 °C	(−)-Geosmin	2.45	2.45	2.40	2.43 ± 0.03 (1%)
	3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
	1*H*-Indole	88.5	82.2	81.9	84.2 ± 3.7 (4%)
	MDMF	42.4	36.7	36.0	38.4 ± 3.5 (9%)

Table S6 Changes in the concentrations of crucial off-flavour compounds in sample MOF2 caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	8.35	8.31	9.09	8.59 ± 0.44 (5%)
	1*H*-Indole	97.1	94.3	114	102 ± 11 (10%)
	MDMF	3.78	3.53	3.61	3.64 ± 0.13 (4%)
125 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	1.60	1.83	1.53	1.65 ± 0.16 (9%)
	1*H*-Indole	273	242	229	248 ± 23 (9%)
	MDMF	3.26	3.66	4.13	3.68 ± 0.44 (12%)
140 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	2.46	2.37	2.59	2.47 ± 0.11 (4%)
	1*H*-Indole	453	356	409	406 ± 49 (12%)
	MDMF	2.10	2.61	2.85	2.52 ± 0.38 (15%)

Table S7 Changes in the concentrations of crucial off-flavour compounds in the reference sample caused by roasting at 110 °C, 125 °C, and 140 °C and processing into cocoa liquor

Temp.	Odorant	Concentration (µg/kg)			
		Exp. 1	Exp. 2	Exp. 3	Mean ± SD
110 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
	1*H*-Indole	48.3	46.7	45.9	47.0 ± 1.2 (3%)
	MDMF	1.46	1.14	1.34	1.32 ± 0.16 (12%)
125 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
	1*H*-Indole	86.2	79.3	82.7	82.7 ± 3.5 (4%)
	MDMF	1.34	1.60	1.40	1.45 ± 0.14 (9%)
140 °C	(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
	3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
	1*H*-Indole	85.4	107	96.3	96.2 ± 10.8 (11%)
	MDMF	1.67	1.69	1.49	1.62 ± 0.11 (7%)

Table S8 Concentrations of crucial off-flavour compounds naturally present in the cocoa liquor before spiking, processing into chocolate mass, and conching

Odorant	Concentration (µg/kg)			
	Exp. 1	Exp. 2	Exp. 3	Mean ± SD
2-Methoxyphenol	67.5	61.5	60.4	63.1 ± 3.8 (6%)
3-Methylphenol	4.41	4.48	4.17	4.35 ± 0.16 (4%)
4-Methylphenol	14.1	14.7	14.2	14.3 ± 0.3 (2%)
3-Ethylphenol	0.386	0.516		0.451 ± 0.092 (20%)
4-Ethylphenol	30.9	30.8	31.4	31.0 ± 0.3 (1%)
3-Propylphenol	0.188	0.220		0.204 ± 0.023 (11%)
(−)-Geosmin	<0.2	<0.2	<0.2	<0.2
3-Methyl-1*H*-indole	<1.0	<1.0	<1.0	<1.0
1*H*-indole	120	114	129	121 ± 8 (6%)
MDMF	<1.0	<1.0	<1.0	<1.0

Table S9 Concentrations of crucial off-flavour compounds after processing spiked cocoa liquor into dark chocolate mass and conching

Odorant	Concentration (µg/kg)			
	Exp. 1	Exp. 2	Exp. 3	Mean ± SD
2-Methoxyphenol	351	338	350	346 ± 7 (2%)
3-Methylphenol	196	190	221	202 ± 16 (8%)
4-Methylphenol	372	390		381 ± 13 (3%)
3-Ethylphenol	120	121	123	121 ± 2 (1%)
4-Ethylphenol	229	239	236	235 ± 5 (2%)
3-Propylphenol	11.8	9.98	10.3	10.7 ± 1.0 (9%)
(−)-Geosmin	4.05	3.95	4.04	4.02 ± 0.05 (1%)
3-Methyl-1*H*-indole	82.4	82.3	86.9	83.9 ± 2.6 (3%)
1*H*-indole	81.6	88.4	83.0	84.3 ± 3.6 (4%)
MDMF	46.8	47.9	48.5	47.8 ± 0.9 (2%)

Table S10 Concentrations of crucial off-flavour compounds after processing spiked cocoa liquor into milk chocolate mass and conching

Odorant	Concentration (µg/kg)			
	Exp. 1	Exp. 2	Exp. 3	Mean ± SD
2-Methoxyphenol	367	375	364	369 ± 6 (2%)
3-Methylphenol	220	223	235	226 ± 8 (4%)
4-Methylphenol	400	407	410	406 ± 5 (1%)
3-Ethylphenol	130	130	124	128 ± 4 (3%)
4-Ethylphenol	234	242	240	239 ± 4 (2%)
3-Propylphenol	11.5	11.6		11.5 ± 0.03 (0.2%)
(−)-Geosmin	4.26	4.23	4.00	4.16 ± 0.14 (3%)
3-Methyl-1*H*-indole	83.4	83.2	86.7	84.4 ± 1.9 (2%)
1*H*-indole	24.7	27.9	26.6	26.4 ± 1.6 (6%)
MDMF	57.6	58.8	56.1	57.5 ± 1.4 (2%)

General remarks to Tables S2–S10

Individual concentration values were rounded to three significant digits for presentation. Mean values were calculated from the unrounded concentrations obtained in the individual experiments and finally rounded to three significant digits. SD stands for standard deviation.