

Supplementary tables

Table S1. Primers used in this study

Name	Primer sequence (5'-3')	Enzyme site	Amplicon size
<i>sdaAA</i> HLarm	F:CGGAATTCAGAGTTTCGGGTTGAAGACGCAGTT R:CGGGGTACCAACTGGCTCCTCCTGACCCTTTCG	<i>EcoR</i> I <i>Kpn</i> I	412bp
<i>cm</i>	F:CGGGGTACCATAGTGACTGGCGATGCTG R:CGCGGATCCTTAAGTTATTGGTATGACTGGTT	<i>Kpn</i> I <i>BamH</i> I	1083bp
<i>sdaAA</i> HRarm	F:CGCGGATCCTATAGCTAACATTAAGGGTATTGGGC R:AACTGCAGCAGCCTGTTTCGTTTTTTCCGTAA	<i>BamH</i> I <i>Pst</i> I	220bp
<i>sdaAA</i> RT-qPCR	F:GTCGTCGCCAATAACGCAAG R:GGGTTCTCCTGCCATTTT		112bp
<i>sdaAA</i> DEDP1	F:TTTACGGGTCTTTTGCG R:AAACCTGCCGTATCCCT		2329bp
<i>katX</i> HLarm	F:CGGAATTCGATTTTCAGTCCGCTTCGTCC R:CGGGGTACCTGGGTTGACCCCTCTTTTTTG	<i>EcoR</i> I <i>Kpn</i> I	337bp
<i>cm</i>	F:CGGGGTACCATAGTGACTGGCGATGCTG R:ACGCGTCGACTTAAGTTATTGGTATGACTGGTT	<i>Kpn</i> I <i>Sal</i> I	1083bp
<i>katX</i> HRarm	ACGCGTCGACAAACAGCGAACAGGAC AACTGCAGGCCAGCACGAAGAAGAATGAATC	<i>Sal</i> I <i>Pst</i> I	437bp
<i>katX</i> RT-qPCR	F:CGGAGTGGGAAGTGTATGCC R:ACAAGCACTCCCGTTCCAA		196bp
<i>katX</i> DEDP	F:TGTTTAGACGCCTTTTCG R:CAGCCGCAATGTGTCTT		2764bp
<i>sdaAA</i>	F:CGGGGTACCGCTGATGACAATAGAGG R:CCCAGCTTTAAATTCCGAGTTCGTGTAA	<i>Kpn</i> I <i>Hind</i> III	1092bp
<i>Erm1</i>	F:AACTGCAGAAATTAAGTCGTAAACCGTGTGC R:CATGCCATGGAGGTGTCACAAGACACTCTTTTT	<i>Pst</i> I <i>Nco</i> I	1200bp
<i>sdaAA</i> DEDP2	F:AGAGTTTCGGGTTGAAGACGCAGTT R:CAGCCTGTTTCGTTTTTTCCGTAA		2498bp
16S rRNA	F:ACTCCTACGGGAGGCAGCAG R:ATTACCGCGGCTGCTGG		197bp

Table S2. Transcriptome data at 37°C and 53°C

	CT53_1	CT53_2	CT53_3	AT37_1	AT37_2	AT37_3	log ₂ FC (BJ3-2 53°C/37°C)
<i>katX</i>	53.28	19.68	35.89	703.96	754.83	840.16	-4.41
<i>sdaAA</i>	78.25	83.05	63.06	483.57	534.06	288.96	-2.50

Table S3. Reverse transcription-quantitative real-time PCR at 37°C and 53°C

	37°C-2^{-ΔΔCT}	53°C-2^{-ΔΔCT}	53°/37°	log₂FC (BJ3-2 53°C/37°C)
<i>katX</i>	1.21	0.03	0.03	-5.16
	1.02	0.04	0.04	-4.82
	1.09	0.03	0.03	-4.99
	0.74	0.04	0.05	-4.28
<i>sdaAA</i>	0.93	0.05	0.05	-4.24
	1.09	0.06	0.06	-4.17
	0.96	0.07	0.07	-3.84
	1.03	0.06	0.06	-4.09

Table S4. Relative content(ug/kg) of volatile compounds in FSs by HS-SPME-GC-O-MS (µg/kg)

No.	Compounds	RIr	RIc	Identification	Concentration (µg/kg)			
					BJ3-2	BJ3-2 <i>AsdaAA</i>	BJ3-2 <i>ΔkatX</i>	BJ3-2 <i>AsdaAAΔkatX</i>
Prazines								
1	Pyrazine	1214	1213	MS/RI	3.80±0.26	3.83±0.30	3.69±0.21	2.70±0.04
2	2-Methylpyrazine	1270	1268	MS/RI	25.52±0.85	30.33±0.95	23.71±1.01	22.44±0.68
3	2,5-Dimethylpyrazine	1326	1326	MS/RI/O/Std	1350.70±53.71	1771.57±9.75	1532.67±26.51	1590.04±11.24
4	2,6-Dimethylpyrazine	1334	1332	MS/RI	112.79±5.67	117.93±13.73	129.39±3.79	103.82±5.56
5	2-Ethyl-5-methylpyrazine	1395	1394	MS/RI/O	13.80±0.77	17.54±0.26	14.81±0.55	15.33±0.27
6	2,3,5-Trimethylpyrazine	1408	1409	MS/RI/O/Std	1859.42±96.23	1477.16±15.35	2085.56±64.72	2121.91±34.18
7	3-Ethyl-2,5-dimethylpyrazine	1447	1445	MS/RI/O/Std	54.75±7.55	53.92±0.29	76.55±5.79	98.29±2.83
8	2,6-Diethylpyrazine	1444	1462	MS/RI	23.11±1.55	12.29±0.33	/	22.02±1.30
9	Tetramethylpyrazine	1478	1478	MS/RI/O	1289.71±79	350.35±12.01	1357.76±82.32	1323.87±55.8
10	2-Ethenyl-6-methylpyrazine	1521	1496	MS/RI	6.50±1.43	13.56±0.55	5.24±0.02	9.55±0.28
11	2-Ethyl-3,5,6-trimethylpyrazine	1491	1521	MS/RI	60.00±10.76	16.93±1.10	71.92±8.38	79.77±3.19
12	2-Ethyl-6-methylpyrazine	1390	1388	MS/RI	/	/	17.00±0.81	11.62±0.16
13	2,3-Dimethyl-5-ethylpyrazine	1460	1462	MS/RI	/	/	24.22±1.96	/
Subtotal					4800.10	3865.41	5342.52	5401.36
Alcohols								
1	Methanethiol	699	-	MS	0.93±0.19	/	/	/
2	3-Methyl-2-pentanol	1202	1194	MS/RI	8.41±2.18	9.68±2.78	10.12±1.56	/
3	2-Heptanol	1316	1315	MS/RI	57.3±7.58	37.26±5.96	45.27±3.96	21.77±2.62
4	3-Methyl-2-buten-1-ol	1317	1317	MS/RI	7.98±1.17	8.86±1.59	8.90±1.12	6.77±0.62
5	1-Hexanol	1348	1349	MS/RI/O	93.62±8.34	105.62±15.89	116.34±9.86	137.99±9.33
6	6-Methyl-2-heptanol	1365	1368	MS/RI	11.47±1.38	10.84±0.59	6.91±0.74	/
7	Trans-3-Hexen-1-ol	1379	1380	MS/RI	6.56±0.46	7.02±0.87	7.57±0.69	6.92±0.44
8	3-Octanol	1385	1388	MS/RI/O	29.47±1.2	31.70±2.34	32.29±0.59	27.38±1.51
9	1-Octen-3-ol	1438	1440	MS/RI/O/Std	320.92±10.19	365.98±25.15	371.14±10.15	385.72±19.81
10	3-Methoxybutanol	814	1510	MS/RI	323.51±56.63	279.78±48.16	273.14±32.92	248.72±87.05
11	2-Nonanol	1525	1516	MS/RI	11.31±1.29	6.08±0.48	3.92±0.71	/
12	(2R,3R)-(-)-2,3-Butanediol	1544	1536	MS/RI	176.18±49.83	77.11±32.07	100.09±28.02	113.05±35.85
13	1-Octanol	1556	1556	MS/RI	1.36±0.19	1.33±0.29	1.31±0.18	2.24±0.21
14	3-Furanmethanol	1679	1654	MS/RI	24.59±3.6	24.95±0.94	25.51±0.73	26.07±1.64
15	Benzyl Alcohol	1872	1875	MS/RI	8.42±1.70	7.24±0.75	7.69±0.96	8.29±0.91
16	Phenylethanol	1912	1913	MS/RI	16.79±3.61	12.85±2.21	16.81±2.8	13.55±1.54
17	3-Methyl-2-butanol	1089	1089	MS/RI	/	12.85±3.46	13.95±2.42	/
18	5-Methyl-2-heptanol	1394	1384	MS/RI	/	/	5.39±0.66	/
19	2,6-Dimethyl-4-heptanol	1509	1470	MS/RI	/	/	8.38±2.01	17.62±3.31
20	Glycidol	755	-	MS	/	2.69±0.81	/	43.23±4.51
21	2-Methylbutanol	1210	1203	MS/RI	/	2.62±0.7	/	1.88±0.21
Subtotal					1098.82	1004.46	1054.73	1061.20
Acids								
1	Isobutyric acid	1571	1567	MS/RI	229.64±168.75	105.85±96.78	/	/
2	2-Methylbutyric acid	1664	1669	MS/RI/O/Std	414.02±6.45	238.99±2.43	227.32±8.51	168.81±7.28
3	Glacial acetic acid	1452	1456	MS/RI	/	/	/	25.05±21.38

Subtotal					643.66	344.84	227.32	193.86
Ketones								
1	Acetone	834	807	MS/RI	475.72±29.5	511.26±20.29	481.11±37.23	248.17±7.56
2	2-Butanone	905	886	MS/RI	193.25±17.36	241.44±15.41	202.16±19.47	105.97±4.23
3	3-Methyl-2-butanone	929	917	MS/RI/O	69.51±15.06	92.83±15.94	84.28±18.23	57.20±5.84
4	2,3-Butanedione	963	961	MS/RI/O/Std	41.15±8.86	30.19±1.13	87.58±21.56	162.24±17.82
5	2-Pentanone	974	965	MS/RI	76.97±7.19	61.33±5.92	90.02±10.25	68.12±7.49
6	3-Methyl-2-pentanone	1016	1008	MS/RI	56.26±10.4	90.34±18.99	67.36±13.46	33.16±4.04
7	2-Hexanone	1078	1074	MS/RI	3.80±0.27	4.09±0.68	5.01±0.64	3.83±0.28
8	5-Methyl-2-hexanone	1141	1137	MS/RI	123.04±18.43	108.72±24.67	156.54±31.08	89.63±9.06
9	2-Heptanone	1180	1183	MS/RI/O/Std	78.16±41.36	118.19±7.32	184.64±36.97	144.37±11.12
10	6-Methyl-2-heptanone	1237	1237	MS/RI	34.44±0.37	46.09±1.05	42.21±4.27	31.45±1.06
11	3-Octanone	1254	1254	MS/RI/O/Std	82.08±23.41	52.46±44.76	119.98±7.55	82.76±14.63
12	2-Octanone	1294	1284	MS/RI/O	54.06±15.49	28.69±5.48	25.76±9.94	
13	2-Nonanone	1366	1340	MS/RI/O	25.21±2.26	16.02±1.2	28.73±2.47	19.75±1.97
14	1-Phenyl-2-propanone	1710	1730	MS/RI	3.19±0.31	3.08±0.32	3.72±0.4	/
15	5-Nonen-2-one	1435	1427	MS/RI	/	/	11.28±1.41	/
16	3-Hydroxy-3-methyl-2-butanone	1243	1241	MS/RI	/	/	/	94.21±13.94
17	3-Hydroxy-2-butanone	1286	1285	MS/RI	/	/	/	643.37±39.89
Subtotal					1316.84	1404.73	1590.38	1784.23
Esters								
1	Methyl acetate	839	817	MS/RI	73.54±15.97	91.68±5.64	87.27±10.17	127.11±16.87
2	Methyl propionate	906	892	MS/RI	3.24±0.15	3.53±0.10	2.61±0.20	2.41±0.05
3	Methyl isobutyrate	921	909	MS/RI	12.76±2.73	12.72±0.94	11.89±3.21	8.13±0.73
4	Methyl 2-methylbutyrate	1007	999	MS/RI/O/Std	14.2±2.24	27.33±4.5	13.79±1.92	12.11±2.06
5	Gamma-caprolactone	1696	1709	MS/RI	5.22±0.97	/	/	/
6	Methyl phenylacetate	1758	1760	MS/RI	58.58±12.28	29.23±4.48	39.32±5.48	/
7	Ethyl acetate	889	871	MS/RI	/	0.87±0.13	/	/
8	Ethyl isobutyrate	1004	953	MS/RI	/	2.31±0.55	/	/
9	Methyl tiglate	1188	1191	MS/RI	/	1.39±0.00	/	1.27±0.08
10	S-methyl 3-methylbutanethioate	1225	1226	MS/RI	/	1.90±0.35	/	/
11	Gamma-butyrolactone	1643	1633	MS/RI	/	2.18±2.06	/	/
12	Hexyl acetate	1275	1269	MS/RI	/	/	/	4.49±1.09
Subtotal					167.54	173.14	154.88	155.52
Others								
1	Carbon disulfide	745	-	MS	2.77±0.58	2.51±0.75	4.35±0.38	4.37±0.08
2	3-Methylfuran	851	878	MS/RI	0.69±0.12	1.30±0.11	0.92±0.18	0.75±0.08
3	Methyl ether	524	921	MS/RI	15.92±1.23	20.64±1.07	14.85±0.78	11.94±0.24
4	2-Ethylfuran	949	938	MS/RI/O/Std	7.58±0.09	12.37±2.50	15.40±6.25	10.77±0.64
5	Pyridine	1191	1189	MS/RI	3.23±0.23	3.05±0.22	2.97±0.16	2.09±0.41
6	2,4,5-Trimethyloxazole	1200	1200	MS/RI/O/Std	86.65±4.77	21.02±1.59	89.89±8.85	91.33±5.42
7	2-Pentylfuran	1228	1229	MS/RI/O/Std	7.80±0.56	11.49±2.34	16.37±5.96	13.93±4.24
8	Furan, 2-(2-pentenyl)-, (E)-Ethylene glycol monobutyl ether	1282	1297	MS/RI	0.24±0.08	0.25±0.04	0.25±0.09	0.26±0.10
9		1402	1403	MS/RI	4.07±3.48	/	/	/
10	2-Methyl-5-propionylfuran	1670	1686	MS/RI	1.42±0.15	/	/	/
11	Isobutyramide	1785	1798	MS/RI	7.46±4.24	2.63±1.86	4.71±3.06	/

12	Guaiacol	1859	1858	MS/RI/O/Std	28.02±3.34	42.29±5.36	30.47±3.77	40.17±0.96
13	2,4-Di-tert-butylphenol	2330	1970	MS/RI	160.71±149.49	283.49±53.93	279.01±71.02	65.73±10.78
14	Phenol	2000	1998	MS/RI	14.29±2.1	22.19±2.00	13.07±1.60	16.20±1.47
15	Dimethyl disulfide	1085	1065	MS/RI	/	1.72±0.07	1.61±0.34	/
16	Benzaldehyde	1529	1528	MS/RI	/	/	15.07±11.64	4.09±1.14
17	2-Methylpyrrole	1537	1549	MS/RI	/	/	0.49±0.17	/
18	Methyl sulfide	773	-	MS	/	0.36±0.03	/	/
19	Acetonitrile	1003	989	MS/RI	/	/	/	0.77±0.07
Subtotal					340.85	425.31	489.43	262.4

RIr: Retention indices were obtained from the flavornet database (<https://webbook.nist.gov/chemistry/>).

RIc: Retention index calculated of compounds on DB-WAX Column (60 m x 0.25 mm x 0.25 µm) with a homologous series of n-alkanes (C7-C30).

Identification: MS: mass spectrum comparison using NIST 20.0 database. RI: retention index in agreement with literature value O: the aroma sensed by human nose at the sniffing detector. Std: confirmed by authentic standards.

-: RI cannot be calculated.

/: Not detected in FSs.

Table S5. Data used in the aroma addition experiment

No.	Compounds	Concentration (µg/kg)		Difference in concentration (µg/kg)	Difference in content of 1.5 g FSs (µg)	Concentration of standard compound (µg/µL)	Amount of aroma compound added to 1.5 g FSs (µL)
		BJ3-2	BJ3-2Δ <i>sdaAAΔkatX</i>				
1	2-Ethylfuran	7.5797	10.7720	3.1923	0.0048	0.008	0.6000
2	2,3-Butanedione	41.4820	162.2404	120.7584	0.1811	0.078	2.3218
3	Methyl 2-methylbutyrate	14.2004	12.1122	-2.0882	-0.0031	0.005	-0.6200
4	2,4,5-Trimethyloxazole	86.6476	91.3328	4.6852	0.007	0.029	0.2414
5	2-Pentylfuran	7.8015	13.9347	6.1332	0.0092	0.009	1.0222
6	3-Octanone	82.0797	82.7601	0.6804	0.001	0.004	0.2500
7	2-Heptanone	78.1556	144.3657	66.2101	0.0993	0.106	0.9368
8	2,5-Dimethylpyrazine	1350.7047	1590.0402	239.3355	0.359	0.421	0.8527
9	2,3,5-Trimethylpyrazine	1859.4227	2191.9105	332.4878	0.4987	0.053	9.4094
10	1-Octen-3-ol	320.9210	385.7250	64.8040	0.0972	0.080	1.2150
11	3-ethyl-2,5-methylpyrazine	54.7452	98.2947	43.5495	0.0653	0.032	2.0406
12	2-Methylbutyric acid	470.2698	225.0638	-245.206	-0.3678	0.303	-1.2139
13	Guaiacol	28.0249	40.1737	12.1488	0.0182	0.014	1.3000

Difference in concentration (µg/kg): The difference in concentration is the concentration of the compounds in the FS with BJ3-2Δ*sdaAAΔkatX* minus the concentration of the compounds in the FS with BJ3-2.

Difference in content of 1.5 g FSs (µg/kg): The difference in content for 1.5 g of FS is the difference in concentration multiplied by 0.0015 kg.

Amount added in 1.5 g FSs (µL): The amount added is the difference in content of 1.5 g FS divided by the concentration of standard compounds. Positive sign indicates addition to BJ3-2, negative sign indicates addition to BJ3-2Δ*sdaAAΔkatX*.