

Supplementary Material

Characterization of Plant Volatiles Reveals Distinct Metabolic Profiles and Pathways among 12 Brassicaceae Vegetables

Yu Liu ¹, Hui Zhang ¹, Shivshankar Umashankar ¹, Xu Liang ¹, Hui Wen Lee ¹, Sanjay Swarup ¹ and Choon Nam Ong ^{1,2,*}

¹ NUS Environment Research Institute, National University of Singapore, Singapore 117411, Singapore; eriliu@nus.edu.sg (Y.L.); erizh@nus.edu.sg (H.Z.); shivshankar.nus@gmail.com (S.U.); eriliaxu@nus.edu.sg (X.L.); erilhwl@nus.edu.sg (H.W.L.); sanjay@nus.edu.sg (S.S.)

² Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117549, Singapore

* Correspondence: ephocn@nus.edu.sg; Tel.: +65-6516-4982

Table legend:

Table S1. Identified VOCs by GC-Q-TOF/MS.

Figure legends:

Figure S1. Diagram of the sampling device.

Figure S2. Evaluation of QCs reproducibility through PCA analysis.

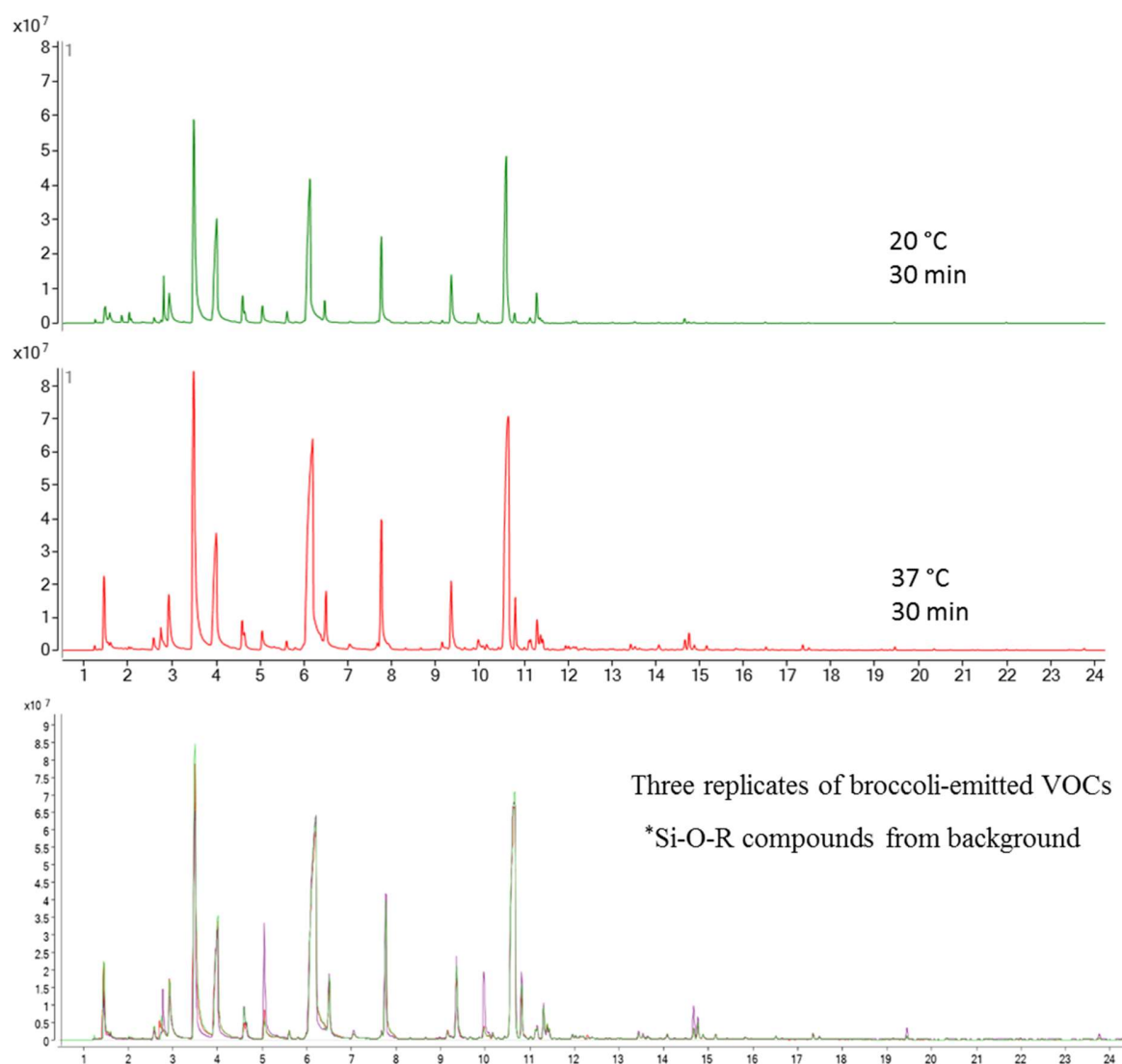
Figure S3. Representative chromatograms of VOCs emitted from broccoli, (a) comparison of extraction temperature, (b) comparison of extraction time, (c) evaluation of addition of water, and (d) the chromatogram under the optimized conditions.

Figure S4. Network plot of Spearman correlations of VOCs in 12 Brassicaceae vegetables.

Table S1. VOCs identified by GC-Q-TOF/MS.

Compound	RT, min	RI ^a	Mono MW	Formula	CAS	Group	VIP ^b	<i>p</i> -Value ^c
3-Decyne	10.47	1001	138.1408	C ₁₀ H ₁₈	2384-85-2	Alkane	1.4	5.86×10 ⁻⁶
Nonadecane	22.17	1407	268.313	C ₁₉ H ₄₀	629-92-5	Alkane	1.1	8.31×10 ⁻⁶
Phenylethyl Alcohol	14.03	1116	122.0731	C ₈ H ₁₀ O	60-12-8	Alcohol	1.1	7.83×10 ⁻⁶
1-Penten-3-ol	2.67	<800	86.0732	C ₅ H ₁₀ O	616-25-1	Alcohol	1.1	2.29×10 ⁻⁵
3-(Z)-Hexenol	6.22	856	100.0188	C ₆ H ₁₂ O	928-96-1	Alcohol	1.1	2.83×10 ⁻⁵
2-(E)-Hexenol	6.52	867	100.0188	C ₆ H ₁₂ O	928-95-0	Alcohol	1.0	5.60×10 ⁻⁶
1-Hexanol	6.6	870	100.0188	C ₆ H ₁₂ O	111-27-3	Alcohol	1.1	6.12×10 ⁻⁶
2-(E)-Hexenal	6.13	852	98.0731	C ₆ H ₁₀ O	6728-26-3	Aldehyde	1.2	7.86×10 ⁻⁶
3-Hexenal	4.72	<800	98.0731	C ₆ H ₁₀ O	4440-65-7	Aldehyde	1.2	8.69×10 ⁻⁶
2,4-(E,E)-Hexadienal	7.82	914	96.0575	C ₆ H ₈ O	142-83-6	Aldehyde	1.3	3.84×10 ⁻⁶
(E)-4-Oxohex-2-enal	9.39	965	112.0524	C ₆ H ₈ O ₂	2492-43-5	Aldehyde	1.3	7.79×10 ⁻⁶
2-Hexenol acetate	11.05	1019	142.0994	C ₈ H ₁₄ O ₂	10094-40-3	Ester	1.24	1.75×10 ⁻⁵
(3Z)-3-Hexenyl acetate	10.75	1010	142.0993	C ₈ H ₁₄ O ₂	1708-82-3	Ester	1.6	1.35×10 ⁻⁴
Hexyl acetate	10.98	1017	144.1150	C ₈ H ₁₆ O ₂	142-92-7	Ester	1.3	8.38×10 ⁻⁶
(4E)-4-Hexenyl propionate	13.6	1102	156.1150	C ₉ H ₁₆ O ₂	-	Ester	1.2	3.57×10 ⁻⁶
(4E)-4-Hexenyl butyrate	14.93	1147	170.1307	C ₁₀ H ₁₈ O ₂	69727-41-9	Ester	1.3	1.45×10 ⁻⁵
(4E)-4-Hexenyl hexanoate	20.58	1347	198.1620	C ₁₂ H ₂₂ O ₂	88552-98-1	Ester	1.3	6.28×10 ⁻⁶
Pentyl acetate	7.9	917	130.0993	C ₇ H ₁₄ O ₂	628-63-7	Ester	1.3	2.14×10 ⁻⁵
Eucalyptol	11.54	1035	154.1358	C ₁₀ H ₁₈ O	470-82-6	Monoterpene	1.2	7.08×10 ⁻⁶
3-Carene	8.47	936	136.1252	C ₁₀ H ₁₆	13466-78-9	Monoterpene	1.0	1.38×10 ⁻⁵
p-Cymene	11.32	1028	134.1095	C ₁₀ H ₁₄	99-87-6	Monoterpene	1.4	1.26×10 ⁻⁵
Camphene	8.97	1052	136.1252	C ₁₀ H ₁₆	79-92-5	Monoterpene	1.2	6.12×10 ⁻⁶
β-Pinene	10.24	993	136.1252	C ₁₀ H ₁₆	127-91-3	Monoterpene	1.3	7.83×10 ⁻⁶
D-Limonene	11.46	1033	136.1252	C ₁₀ H ₁₆	5989-27-5	Monoterpene	1.6	5.94×10 ⁻⁶
γ-Terpinene	12.36	1061	136.1252	C ₁₀ H ₁₆	99-85-4	Monoterpene	1.2	1.12×10 ⁻⁵
Allyl Isothiocyanate	6.9	882	99.0142	C ₄ H ₅ NS	57-06-7	Isothiocyanate	1.7	3.82×10 ⁻⁶

3-Butenyl isothiocyanate	9.905	982	113.0299	C ₅ H ₇ NS	3386-97-8	Isothiocyanate	1.7	4.38×10 ⁻⁶
3-Methylbutyl isothiocyanate	12.29	960	129.0612	C ₆ H ₁₁ NS	628-03-5	Isothiocyanate	1.4	4.69×10 ⁻⁶
4-Methylpentyl isothiocyanate	15.41	1163	143.0769	C ₇ H ₁₃ NS	17608-07-0	Isothiocyanate	1.1	5.98×10 ⁻⁶
Pentyl isothiocyanate	13.47	1098	129.0612	C ₆ H ₁₁ NS	629-12-9	Isothiocyanate	1.3	6.20×10 ⁻⁶
Hexyl isothiocyanate	16.51	1200	143.0769	C ₇ H ₁₃ NS	4404-45-9	Isothiocyanate	1.1	3.16×10 ⁻⁶
Heptyl isothiocyanate	18.33	1264	157.0925	C ₈ H ₁₅ NS	4426-83-9	Isothiocyanate	1.2	4.22×10 ⁻⁶
3-Methylthiopropyl isothiocyanate	19.64	1311	147.0176	C ₅ H ₉ NS ₂	505-79-3	Isothiocyanate	1.5	7.83×10 ⁻⁶
4-Methylthio-3-butenyl isothiocyanate	22.46	1419	159.0176	C ₆ H ₉ NS ₂	51598-96-0	Isothiocyanate	1.2	6.34×10 ⁻⁶
Phenethyl isothiocyanate	23.7	1467	163.0456	C ₉ H ₉ NS	2257-09-2	Isothiocyanate	1.3	5.53×10 ⁻⁶
Dimethyl disulfide	3.58	<800	93.9911	C ₂ H ₆ S ₂	624-92-0	Sulphur compounds	1.1	5.39×10 ⁻⁶
Dimethyl trisulfide	9.51	969	125.9631	C ₂ H ₆ S ₃	3658-80-8	Sulphur compounds	1.0	7.60×10 ⁻⁶
1-(Methylsulfanyl)-1,3-butadiene	5.86	842	100.0347	C ₅ H ₈ S	10574-97-7	Sulphur compounds	1.0	2.26×10 ⁻⁶
3-Methylisothiazole	10.64	1006	99.0143	C ₄ H ₅ NS	693-92-5	Sulphur compounds	1.5	9.45×10 ⁻⁶
4,5-Dimethylthiazole	14.245	1124	113.0299	C ₅ H ₇ NS	3581-91-7	Sulphur compounds	1.6	4.12×10 ⁻⁶
4-Propylthiazole	13.01	1083	127.0456	C ₆ H ₉ NS	41981-60-6	Sulphur compounds	1.6	3.30×10 ⁻⁵
3-Butenenitrile	2.38	<800	67.0422	C ₄ H ₅ N	109-75-1	Nitrile	1.5	5.21×10 ⁻⁶
3-Pentenitrile	3.955	<800	81.0578	C ₅ H ₇ N	4635-87-4	Nitrile	1.4	1.01×10 ⁻⁵
2-Methylbutanenitrile	5.81	840	83.0735	C ₅ H ₉ N	18936-17-9	Nitrile	1.2	1.51×10 ⁻⁴



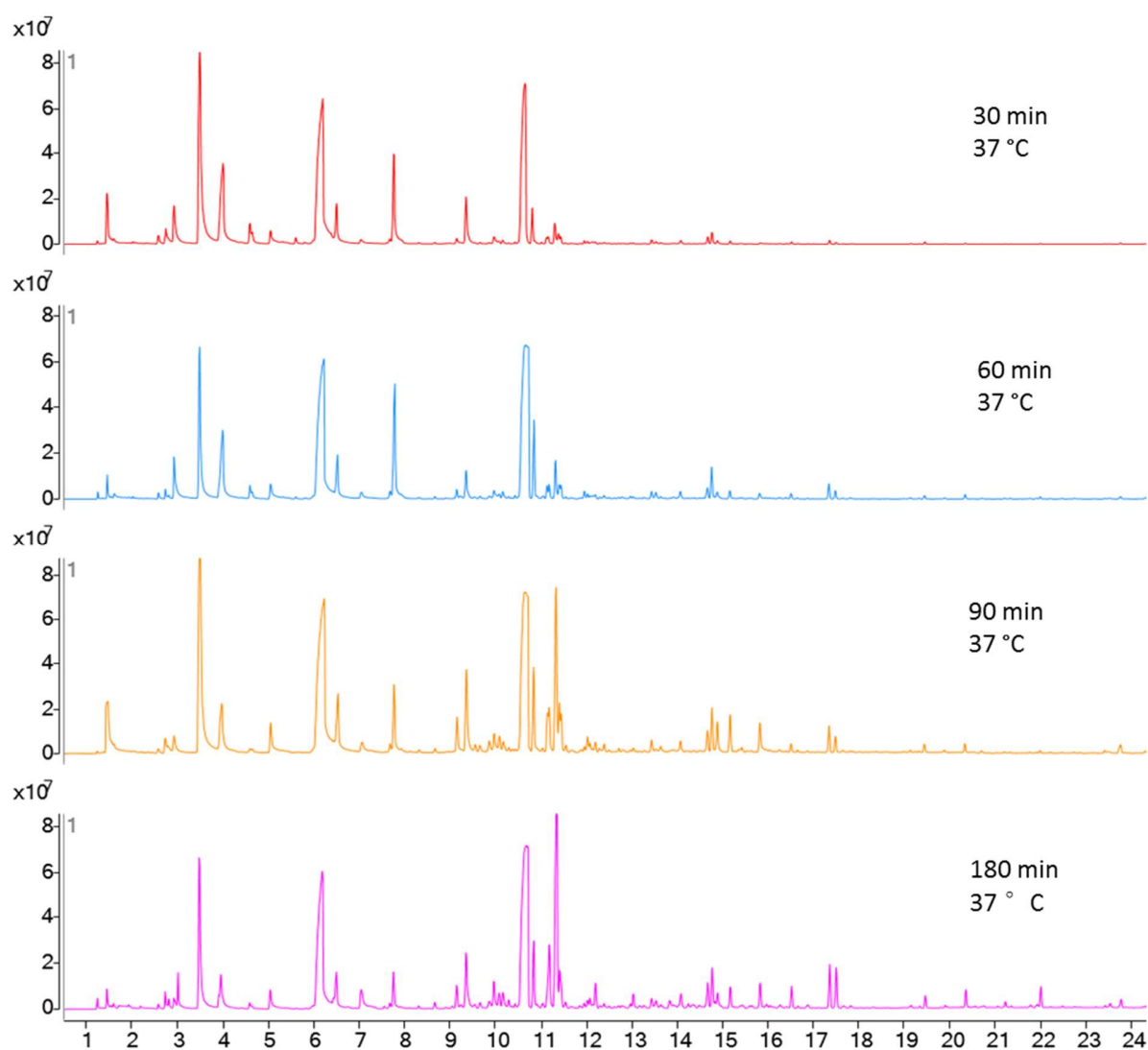
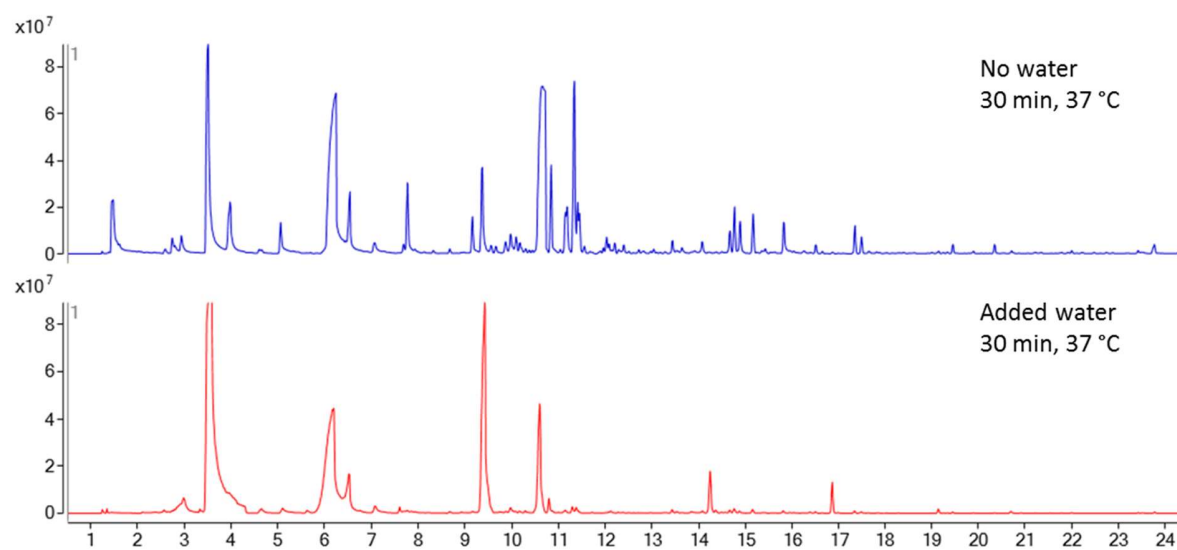
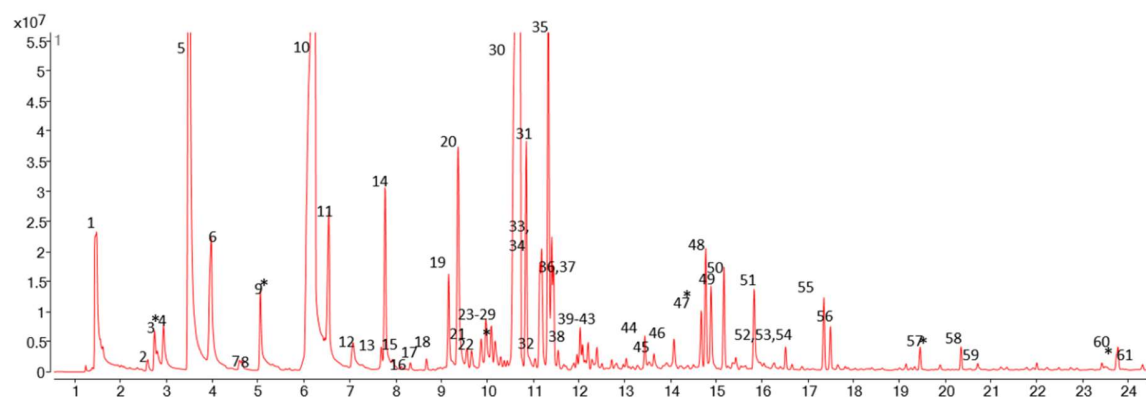


Figure S1b. Comparison of extraction time.



23

Figure S1c. Evaluation of addition of water.

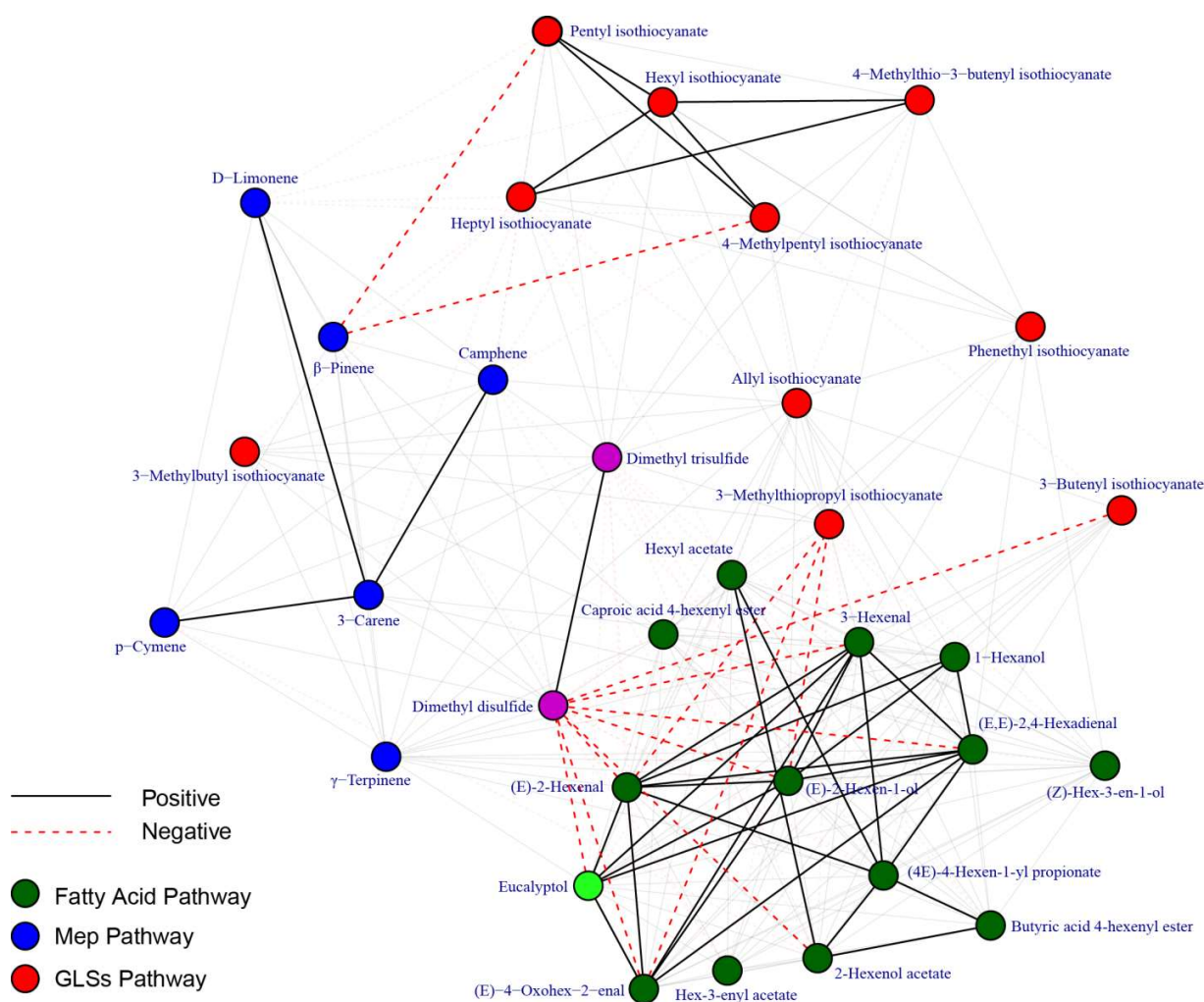


- | | | | | |
|-----------------------------|--|--|--|---|
| 1. Acetone, | 13. 2-Penten-1-ol, acetate, (Z)-, | 26. Benzene, 1-ethyl-3-methyl-, | 38. Undecane, 3,6-dimethyl-, | 51. Levomenthol, |
| 2. 1-Penten-3-ol, | 14. Acetic acid, pentyl ester, | 27. Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, | 39. Propanoic acid, 2-methyl-, pentyl ester, | 52. Cyclopentane, 1-hexyl-3-methyl-, |
| 3. Si-O-R from SPME fiber | 15. 4-Penten-1-ol, 3-methyl-, acetate, | [1R-(1a,2a,5a)]-, | 40. Heptane, 5-ethyl-2,2,3-trimethyl-, | 53. Nonadecane, |
| 4. Methyl isothiocyanate, | 17. 3-Carene, | 28. Octane, 2,6,6-trimethyl-, | 41. γ -Terpinene, | 54. 8-Dodecenol, |
| 5. Disulfide, dimethyl, | 18. 3-Decyne, | 29. Tetradecane, | 42. 1,2,6-Hexanetriol, | 55. Pentanoic acid, 4-hexen-1-yl ester, 56. |
| 6. 1-Pentanol | 19. Octane, 2,2,6-trimethyl-, | 30. 4-Hexen-1-ol, (4E)-, acetate, | 43. Undecane, 3-methyl-, | n-Valeric acid cis-3-hexenyl ester, |
| 7. 3-Hexenal, | 20. Dimethyl trisulfide, | 31. Acetic acid, hexyl ester, | 44. Propanoic acid, 4-hexen-1-yl ester, 45. | 57. Si-O-R from SPME fiber |
| 8. 1,2-Methylcyclopentanol, | 21. Pentane, 2,2,3,4-tetramethyl-, | 32. Benzene, 1,2,3-trimethyl-, | 3-Nonen-1-ol, (Z)-, | 58. Dodecanoic acid, 2-hexen-1-yl ester, |
| 9. Si-O-R from SPME fiber | 22. Tetradecane, 1-chloro-, | 33. Heptane, 4-ethyl-2,2,6,6-tetramethyl-, | 46. Isophorone, | 59. 5-Keto-2,2-dimethylheptanoic acid, |
| 10. 3-Hexen-1-ol, (E)-, | 23. Heptane, 2,2,3,5-tetramethyl-, | 34. β -Cymene, | 47. Si-O-R from SPME fiber | ethyl(ester), |
| 11. 1-Hexanol, | 24. Si-O-R from SPME fiber | 35. D-Limonene, | 48. Butanoic acid, 4-hexen-1-yl ester, | 60. Si-O-R from SPME fiber |
| 12. p-Xylene, | 25. Decane, 2,6,7-trimethyl-, | 36. Eucalyptol, | 49. Camphor, | 61. Guaia-1(10),11-diene, |
| | | 37. Hexane, 3,3-dimethyl-, | 50. l-Menthone, | |

24

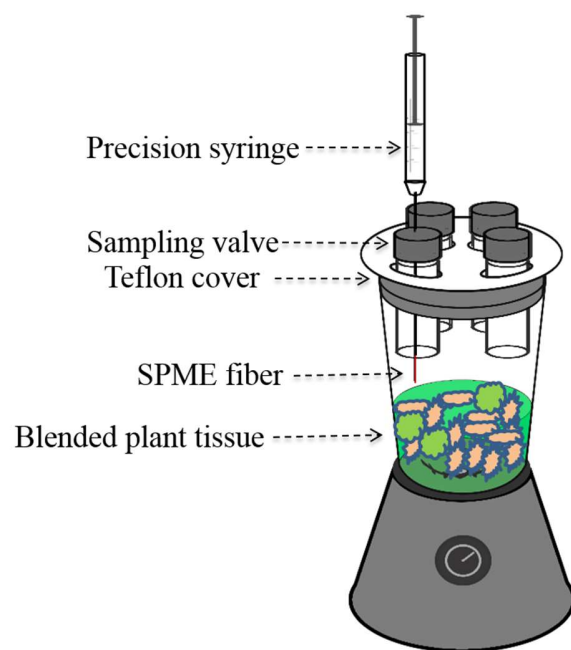
Figure S1d. The chromatogram under the optimized conditions.

Figure S1. Representative chromatograms of VOCs emitted from broccoli: (a) comparison of extraction temperature, (b) comparison of extraction time, (c) evaluation of addition of water, and (d) the chromatogram under the optimized conditions.



Note: Significant correlations ($p < 0.05$) were plotted between VOCs. The positive correlation with a correlation coefficient higher than 0.8 is shown by the black solid line. The negative correlation with a correlation coefficient lower than -0.5 is shown by the red dashed line.

Figure S2. Network plot of Spearman correlations of VOCs in 12 Brassicaceae vegetables.



26

Figure S3. Diagram of the sampling device.

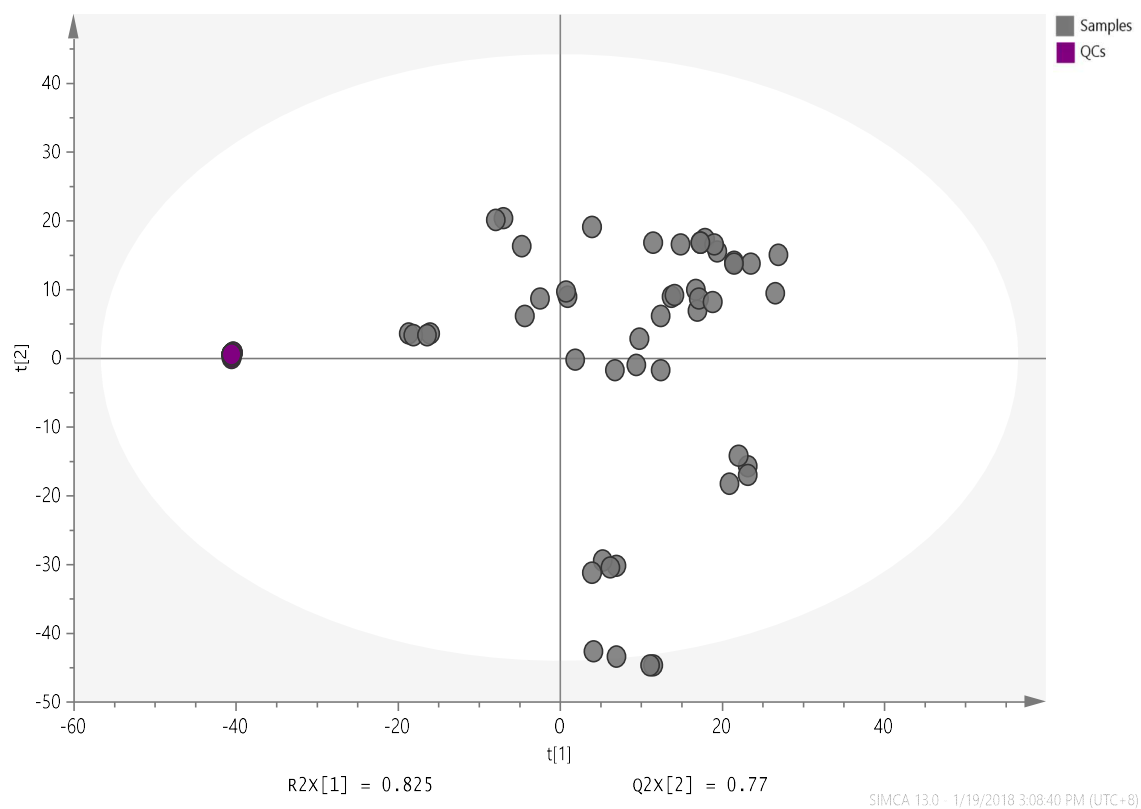


Figure S4. Evaluation of QCs' reproducibility through PCA analysis.