

1 **Supplementary material**

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4 **Statistical Evaluation to Validate Matrix-Matched Calibration for**
5 **Standardized Beany Odor Compounds Quantitation in Yellow Pea Flour**
6 **Using HS-SPME-GC-MS**

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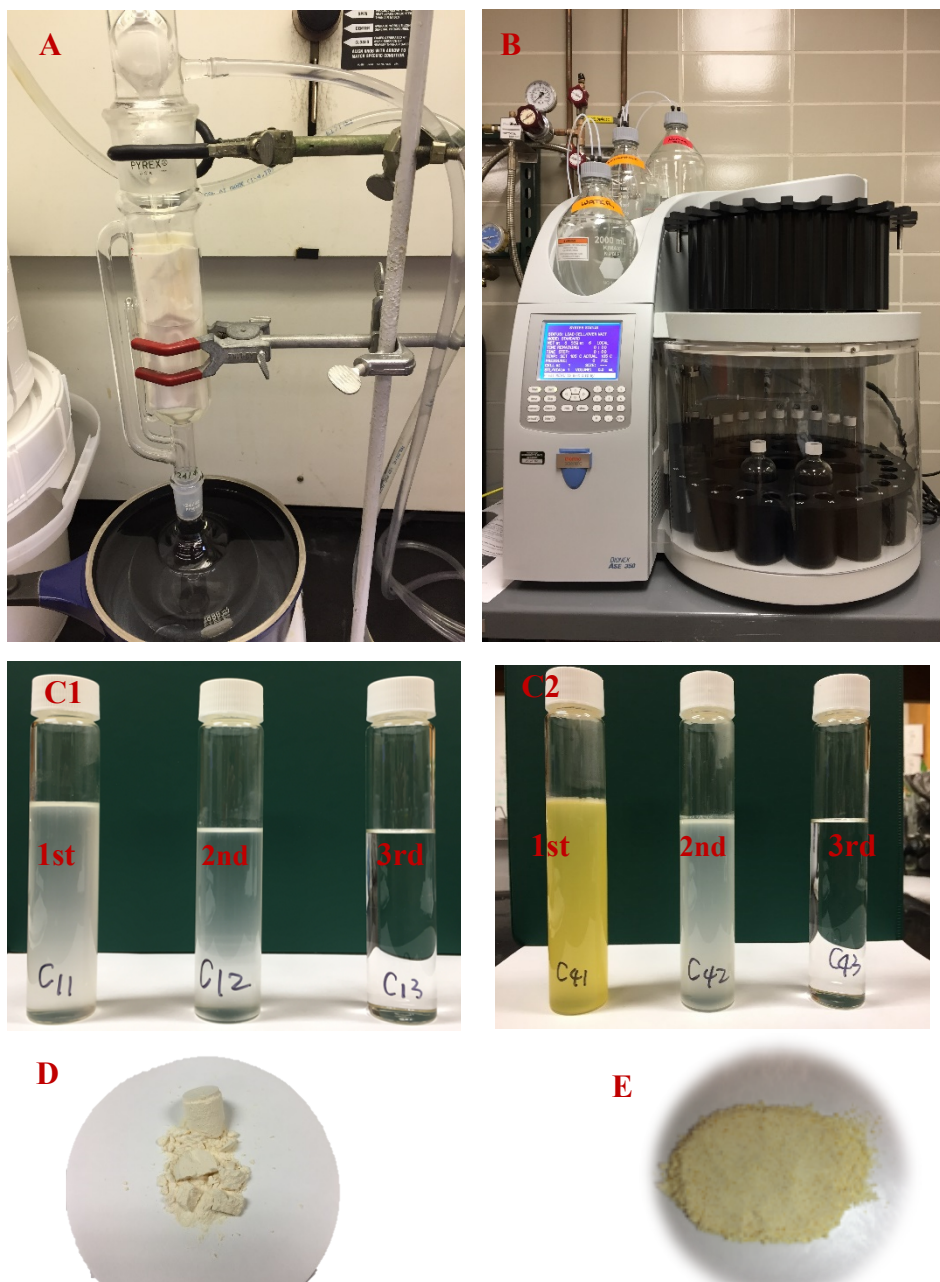
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17 **1. Figures and tables**

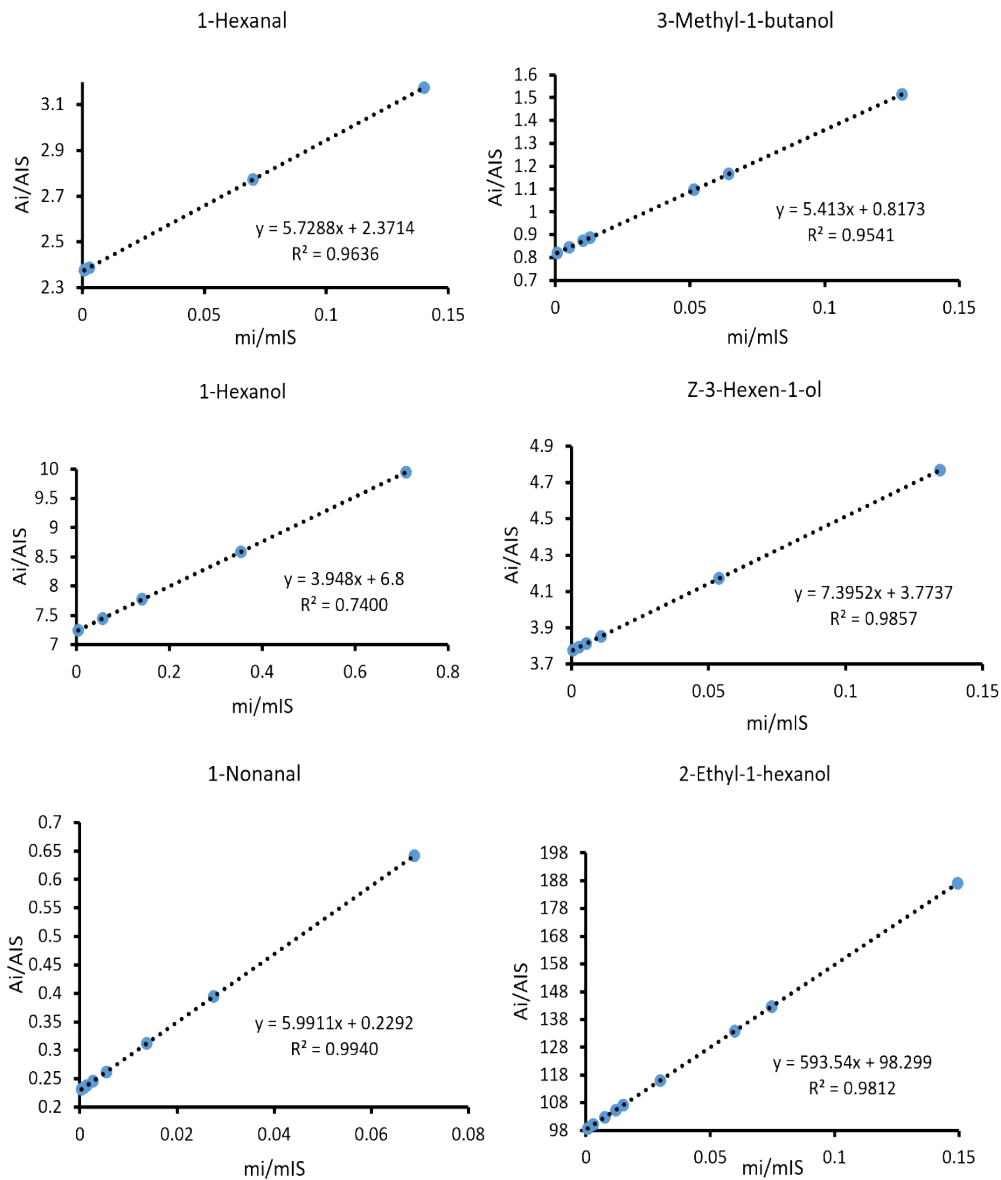
18 Fig. S1 (A) Soxhlet extraction, (B) Accelerated dichloromethane extraction (ASEDCM),

19 Raffinate from (C1) three-cycle of accelerated dichloromethane extraction of starch & (C2)

20 three-cycle of accelerated dichloromethane extraction of yellow pea, (D) Deodorized matrix-

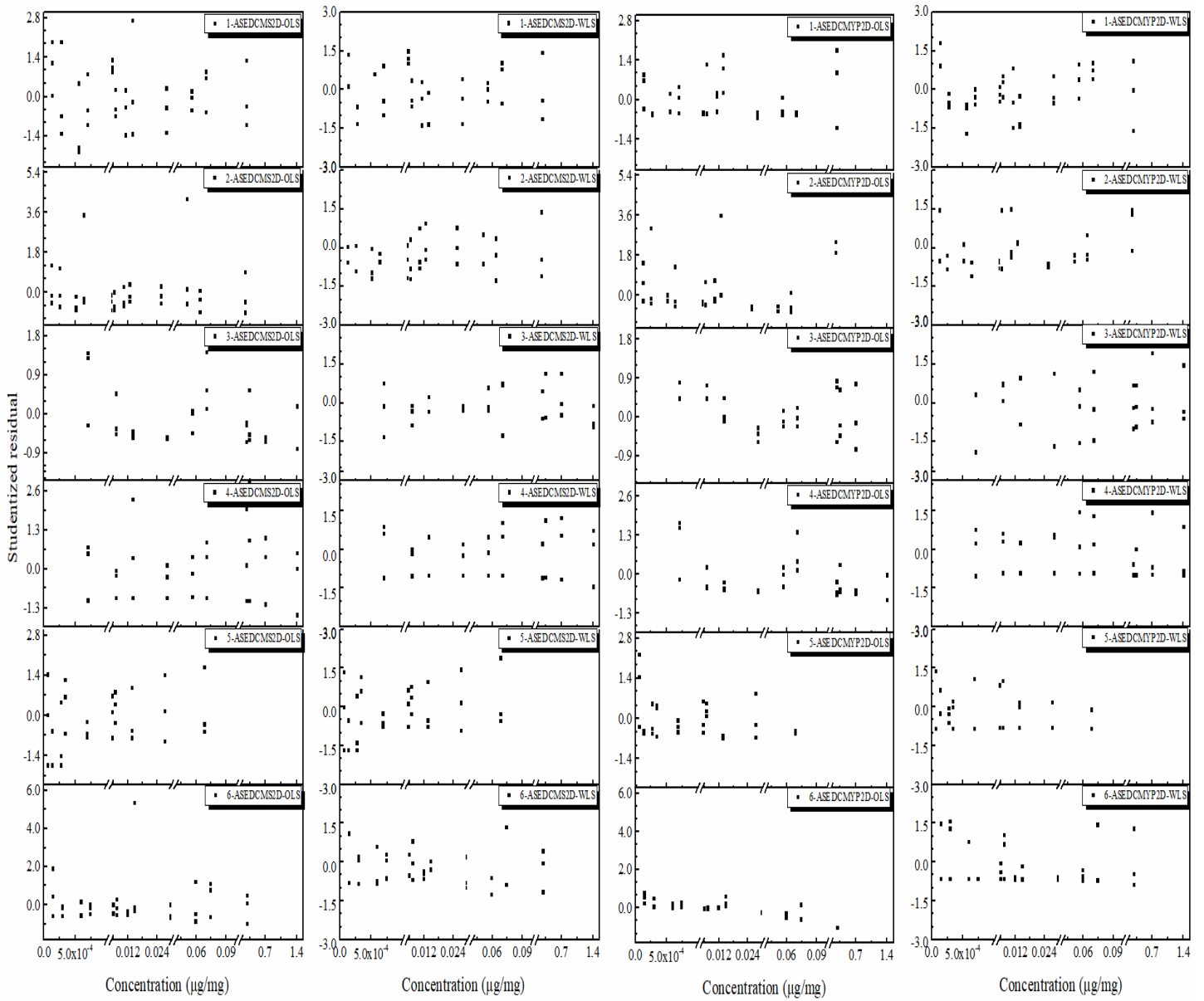
21 matched yellow pea flour from accelerated dichloromethane extraction (ASEDCMYP), and

22 (E) Native yellow pea flour.



25 Fig. S2 External standard calibration curves of six beany odor makers established in methanol

26 via weighted least square regression



28 ■ 1-6 denote as beany odor markers 1-hexanal, 3-methyl-1-butanol, 1-hexanol, (3Z)-hexen-1-ol, 1-nonanal, and 2-

29 ethyl-1-hexanol, respectively.

30 Fig. S3 Residual plots of six beany odor makers in two deodorized matrix-matched flours via

31 ordinary least square (OLS) and weighted least square (WLS) regression.

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Table S1. Literature investigation on beany odor quantification in pea cultivars

Literature reported	Compound						Pea details	Processing	Quantification methods
	1-hexanal	3-methyl-1-butanol	1-hexanol	(3Z)-hexen-1-ol	1-nonanal	2-ethyl-1-hexanol			
1	√	√	√	√	√		Commercial green pea (GP)	Unblanched GP	No
2		√	√	√			GP grown at Richmond, N.S.W.	Blanched GP	No
3	√	√	√	√	√		GP variety <i>medullare</i> Alef in Denmark	Blanched GP	Semi-quantification with IS
4	√						6 YP, 3 GP cultivars in Saskatchewan	Crop years 2005-2007	Ratio of relative peak area
5	√	√	√	√	√		Commercial pea flour	SAFE/SPME extraction	Semi-quantification with IS
6	√	√	√	√	√		Commercial YP	Germination from 1 to 6 days	Calibration curves in deodorized starch
7	√		√		√		YP Zhong-wan variety	Crop year 2017, raw and roasted YP	Calibration curves in deodorized YP flour
8	√	√	√		√	√	YP Agassize and Trapeze cultiar	Crop year 2018	Ratio of relative peak area
9	√	√	√	√	√	√	Commercial YP	Different milling configurations	Calibration curves in deodorized YP flour

34 √ indicates the compound has been reported in literature.

35 GP: green pea, YP: yellow pea, IS: internal standard, SAFE: solvent assisted flavor evaporation; SPME: solid phase microextraction.

36

37 Table S2. Preparation of beany odor mixed stock solutions and corresponding dilution factors

		Concentration of each beany odor standard in mixed SS/ mg/mL					
Compound		1-hexanal	3-methyl-1-butanol	1-hexanol	(3Z)-hexen-1-ol	1-nonanal	2-ethyl-1-butanol
Weight/g		0.1063	0.0977	0.1076	0.1020	0.1045	0.1134
Methanol/mL		10	10	10	10	10	10
SS ¹ /mg/mL		10.63	9.77	10.76	10.2	10.45	11.34
Code	DF ²	Concentration of each beany odor standard in diluted mixed solutions / $\mu\text{g/mL}$					
B25	2.50×10^3	0.45	0.41	2.27	0.43	0.22	0.48
B50	5.00×10^3	0.22	0.21	1.13	0.22	0.11	0.24
D	1.00×10^4	0.11	0.10	0.57	0.11	0.06	0.12
A50	1.25×10^4	0.09	0.08	0.45	0.08	0.04	0.10
C25	2.50×10^4	0.05	0.04	0.23	0.04	0.02	0.05
C50	5.00×10^4	0.02	0.02	0.11	0.02	0.01	0.02
B2525	6.25×10^4	0.018	0.016	0.091	0.017	0.009	0.019
E	1.00×10^5	0.011	0.010	0.057	0.011	0.006	0.012
B5025	1.25×10^5	0.009	0.008	0.045	0.009	0.004	0.010
D25	2.50×10^5	0.004	0.004	0.023	0.004	0.002	0.005
D50	5.00×10^5	0.002	0.002	0.011	0.002	0.001	0.002
F	1.00×10^6	0.001	0.001	0.006	0.001	0.0006	0.001
C5025	1.25×10^6	0.0009	0.0008	0.0045	0.0009	0.0004	0.0010
E25	2.50×10^6	0.0005	0.0004	0.0023	0.0004	0.0002	0.0005
E50	1.25×10^8	0.0002	0.0002	0.0011	0.0002	0.0001	0.0002

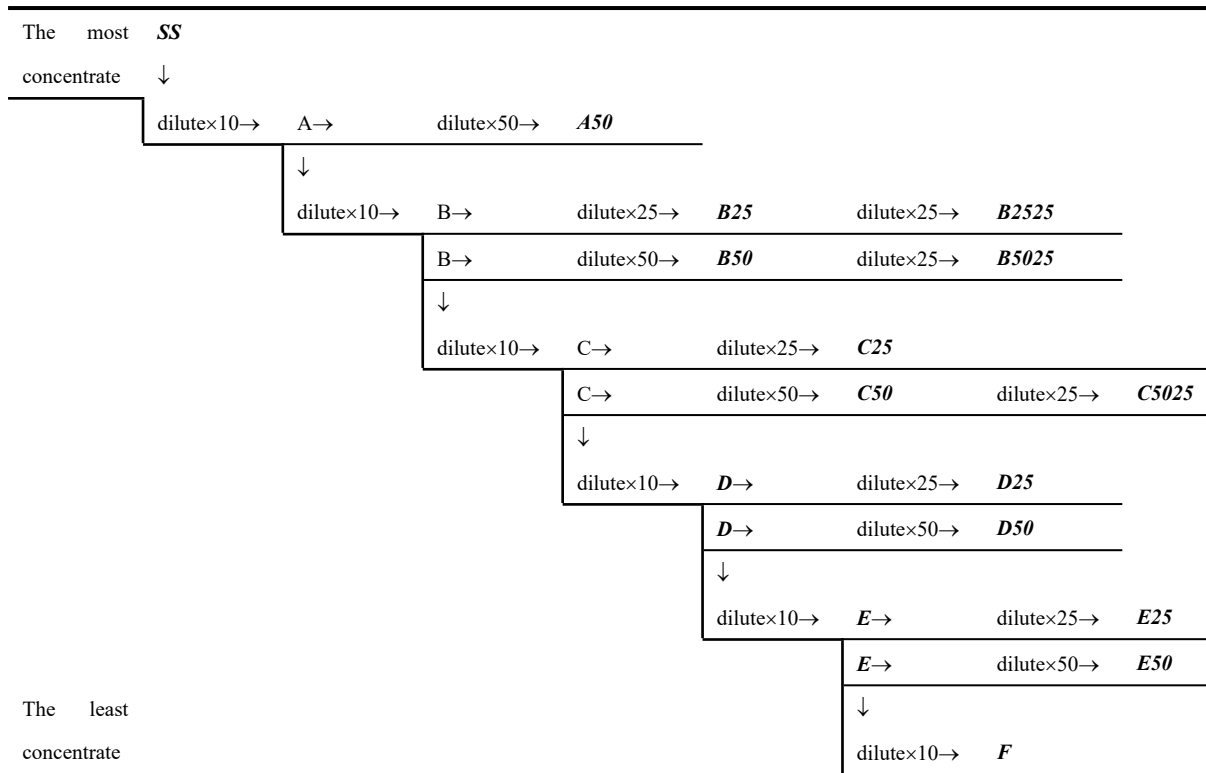
38 1. SS represents stock solution in methanol.

39 2. DF represents dilution factor, diluted with methanol.

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Table S3. Serial dilutions of beany odor mixed stock solutions



42 The codes and concentrations are corresponding to those in Table S2.

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44 **2. Material and methods**

45 2.3 Physical attributes of deodorized matrix-matched flours

46 2.3.1 Surface morphology

47 Surface morphology of deodorized matrix-matched flour from different treatment
48 combinations were observed via JEOL JSM-6300 Scanning Electron Microscope (SEM, JEOL
49 USA, Peabody, MA USA)⁹. The flour samples were adhered to aluminum mounts by carbon
50 tabs and coated with gold using a Balzers SCD 030 sputter coater (BAL-TEC RMC, Tucson,
51 AZ USA). Images were taken at × 1000 magnification with an accelerating voltage of 15 kV.

52 2.3.2 Particle size distribution

53 Particle size distribution (PSD) of deodorized matrix-matched flour from different
54 treatment combinations were determined by a laser light scattering instrument (Mastersizer
55 3000, Malvern Instruments Ltd., Malvern, U.K.) in dry powder sampling module⁹. The particle
56 size was determined from intensity–time fluctuations of the laser light scattered from a sample
57 at a fixed angle of 173°. Measurements were reported as the volume-weight mean diameter (D
58 _[4,3] = $\sum n_i d_i^4 / \sum n_i d_i^3$), where n_i was the number of particles of diameter d_i .

59 2.4 Beany odor analysis

60 2.4.1 beany odor detection

61 The beany odor detection and identification were achieved by headspace-solid phase
62 microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS, 7890B GC
63 system, 5977A mass detector, Agilent Technologies, Santa Clara, CA, USA.) under scanning
64 and selective ion mode (SIM) according to previous study with minor modifications⁹.

65 A SPME fiber coated with DVB/CAR/PDMS (50/30 μm , Supelco Inc. Bellefonte, PA)
66 was applied to extract the volatile compounds. PAL RSI 120 autosampler (CTC Analytics,
67 Zwingen, Switzerland) was employed for the incubation and extraction procedure. An aliquot
68 of 0.5 g YP flour mixed with 2 mL of deionized water was transferred in a 20 mL glass vial
69 capped by aluminum caps with PTFE/silicone septa (diam. \times thickness 20 mm \times 0.105 in,
70 MilliporeSigma, St. Louis, MO, USA). Vials were incubated for 5 min at 60 $^{\circ}\text{C}$ in an
71 autosampler heating block, while agitating at 250 rpm. Thereafter, the SPME fiber needle was
72 inserted into the vial for 60 min to adsorb volatiles at 60 $^{\circ}\text{C}$, which was then transferred into
73 the injector port (250 $^{\circ}\text{C}$) for 3 min desorption.

74 The separation of volatiles was performed on an Agilent Technologies 7890B GC system.
75 The injection port was operated in splitless mode. The liner was Agilent 5190-2293: 900 μL
76 (Splitless, single taper, Ultra Inert) and the purge flow to split vent was 50 mL/min at 1 min.
77 Volatiles were separated on a J&W DB-Wax column (60 m \times 0.25 mm i.d., 0.25 μm film
78 thickness, Agilent Technologies, Santa Clara, CA, USA.). Oven temperature program was set
79 as: initial temperature 40 $^{\circ}\text{C}$, followed by an increase of 45 $^{\circ}\text{C}/\text{min}$ to 85 $^{\circ}\text{C}$, then by 3 $^{\circ}\text{C}/\text{min}$
80 to 135 $^{\circ}\text{C}$ and held for 1 min, next by 1 $^{\circ}\text{C}/\text{min}$ to 160 $^{\circ}\text{C}$ and held for 1 min, afterwards by 9
81 $^{\circ}\text{C}/\text{min}$ to 200 $^{\circ}\text{C}$, and lastly by 45 $^{\circ}\text{C}/\text{min}$ to 250 $^{\circ}\text{C}$ and held for 12 min. Helium carrier gas
82 (purity 99.9999%, Praxair, Fargo, ND, USA.) was used at a flow rate of 2 mL/min. The column
83 effluent was transferred to the 5977A mass detector, operated in electron impact (EI) ionization
84 mode at 70 eV. The temperature of MS detector transfer line was hold at 250 $^{\circ}\text{C}$. The MS
85 quadrupole temperature was 150 $^{\circ}\text{C}$. Ion source temperature was 230 $^{\circ}\text{C}$. Scanning and
86 selecting ion monitoring mode (SIM) was applied with scan time and segments setting from 4

87 to 63 min and scanning m/z from 40 to 350.

88 2.4.2 Identification of beany odor markers

89 The beany odor markers were identified by three methods: i) raw data acquired by GC–
90 MS were analyzed using Agilent MassHunter Qualitative Analysis (B.07.00) coupled with MS
91 library NIST 17. The min match score > 85 was the main criteria applied for validating the
92 entities; ii) to calculate the retention index (RI) of each compound using a series of n-alkanes
93 C8–C40 (MilliporeSigma, St. Louis, MO, USA). Calculated RI was matched with a reference
94 value according to NIST database (<https://webbook.nist.gov>); and iii) standard chemical
95 compounds were applied to further verify the compounds under the aforementioned GC–MS
96 conditions.

97 The retention time (RT) around 9.5 min with a SIM quantifier ion of 56.1 was assigned to
98 1-hexanal (9.5 min-56.1 m/z , **1**). The remaining beany odor makers were: 15.1 min-55.1 m/z (**2**),
99 15.3 min-43 m/z (**3**), 16.1 min-41 m/z (**4**), 16.6 min-56.1 m/z (**5**), 19.6 min-57.1 m/z (**6**), and 12.3
100 min-85 m/z (**IS**), respectively. The detailed information regarding SIM ions parameters is listed
101 below.

Time/min	Group name	Number of ions	Mass1/dwell time/s	Mass2/dwell time/s	Mass3/dwell time/s
9.5	Hexanal	3	41.00/50	44.10/50	56.10*/50
12.3	2-Methyl-3-heptanone	3	57.00/50	85.00*/50	128.00/50
15.1	3-Methyl-1-butanol	3	42.00/50	55.10*/50	70.10/50
15.3	1-Hexanol	3	43.00/50	55.10/50	56.10/50
16.1	(3Z)-Hexen-1-ol	3	41.00*/50	67.00/50	82.10/50
16.6	Nonanal	3	41.00*/50	56.10/50	57.10/50

19.6	2-Methyl-1-hexanol	3	41.00/50	43.00/50	57.10*/50
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102 * represented quantitative SIM ion.

103 **3. Results and discussion**

104 **3.4 Visual Evaluation of Linearity of Calibration Curves**

105 As displayed in Fig. S3, there were strong evidence of heteroskedasticity in the response
106 of each beany odor marker via OLSR in both deodorized matrix-matched flours as the
107 distribution was neither placed randomly alongside zero, nor shown an increasing behavior of
108 the deviations. Similar residual plot results were also reported by Gomes et al. in pesticides
109 quantitation by ESC method¹⁰.

110 Outliers were vastly detected in compound **1, 2, 4, 5, 6** in ASEDCMS2D matrix, whereas
111 they were only noticed in compound **2** and **6** in ASEDCMYP2D matrix. After removing the
112 outliers, the studentized residual by WLSR showed a more discrete and random distribution,
113 especially in ASEDCMYP2D matrix. Take compound **3** as an example, the residual
114 distribution was completely dispersed alongside the concentration, and each replicate was
115 almost discrete from each other in ASEDCMYP2D matrix when regressed by WLSR.
116 Conversely, it showed a declined trend and later fanned out in ASEDCMYP2D matrix with
117 OLSR. Besides that, there seemed to be an interesting phenomenon that the residual
118 distribution patterns of each beany odor marker were more random in ASEDCMYP2D matrix
119 than in ASEDCMS2D matrix with the same WLSR, in which certain distribution patterns of
120 the latter still showed a bit fan-out trend such as compound **5**.

121 **3.6 Beany Odor Compounds Quantitation**

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125 Taking hexanal in ASEDCMYP2D as an example,

$A_i^{56.1}$	A_{IS}^{85}	$A_i^{56.1}/A_{IS}^{85}$	$m_i^{56.1}/m_{IS}^{85}$	$m_i^{56.1}$ -ASEDCMYP/ug	$m_i^{56.1}$ -ASEDCMYP/ug/g	Mean	STD
2520.64	259.74	9.70	0.23	0.37	0.75	1.11	0.51
3193.58	188.80	16.92	0.46	0.74	1.47		

126

127 $A_i^{56.1}$ was the response peak area of SIM ion 56.1 of hexanal reading from the TIC;

128 A_{IS}^{85} was the response peak area of SIM ion 85 of internal standard reading from the TIC;

129 $m_i^{56.1}/m_{IS}^{85}$ was interpolated according to the calibration curve $A_i^{56.1}/A_{IS}^{85} = 31.648 m_i^{56.1}/m_{IS}^{85} + 2.331$;

130 $m_i^{56.1}$ -ASEDCMYP/ug was achieved by multiple the mass of added IS, which was 1.598 μ g;

131 $m_i^{56.1}$ -ASEDCMYP/ug/g was achieved by dividing the applied sample mass of 0.5 g.

132 The final quantitative results were reported by mean \pm std.

133 Taking hexanal in ASEDCMS2D as an example,

$A_i^{56.1}$	A_{IS}^{85}	$A_i^{56.1}/A_{IS}^{85}$	$m_i^{56.1}/m_{IS}^{85}$	$m_i^{56.1}$ -ASEDCMS/ug	$m_i^{56.1}$ -ASEDCMS/ug/g	Mean	STD
2697.71	495.20	5.45	0.50	0.79	1.59	1.47	0.17
2575.21	526.4	4.892	0.42	0.67	1.35		

134 $m_i^{56.1}/m_{IS}^{85}$ was interpolated according to the calibration curve $A_i^{56.1}/A_{IS}^{85} = 7.159 m_i^{56.1}/m_{IS}^{85} + 1.872$;

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