Supporting Information

Evaluation of a quick one-step sample preparation method for the determination of the isotopic fingerprint of rapeseeds – Investigation of the influence of the use of 2,2-Dimethoxypropane (DMP) on C- and H-CSIA by GC-C/Py-IRMS

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Table S- 1: Fatty acid profile of rapeseed (mol%) using 3 different transmethylation mixture

transmethylation mixture		**TM+D, n=3**			**TM-D, n=3**			**TX-D, n=3**			**mean**	**SD**
FAME composition		Fi[a]	mean	SD	Fi[a]	mean	SD	Fi[a]	mean	SD		
Fatty acid composition (mol %)	**C14:0**	1.02	0.1%	0.01%	1.02	0.1%	0.01%	1.02	0.1%	0.00%	**0.1%**	0.02%
	C16:0	1.00	5.5%	0.04%	1.00	5.6%	0.02%	1.00	5.5%	0.07%	**5.5%**	0.06%
	C16:1	1.00	0.4%	0.02%	1.00	0.4%	0.02%	1.00	0.3%	0.00%	**0.3%**	0.05%
	C18:0	0.99	1.7%	0.01%	0.99	1.7%	0.03%	0.99	1.8%	0.09%	**1.7%**	0.09%
	C18:1	1.00	61.6%	0.06%	1.00	61.4%	0.04%	1.00	62.5%	0.12%	**61.8%**	0.61%
	C18:2	0.98	18.2%	0.05%	0.98	18.4%	0.02%	0.98	17.7%	0.02%	**18.1%**	0.36%
	C18:3	1.00	10.0%	0.01%	1.00	10.1%	0.02%	1.00	9.3%	0.02%	**9.8%**	0.47%
	C20:0	0.99	0.5%	0.00%	0.99	0.5%	0.01%	0.99	0.6%	0.00%	**0.5%**	0.05%
	C20:1	1.00	1.2%	0.01%	1.00	1.2%	0.01%	0.96	1.3%	0.01%	**1.2%**	0.04%
	C22:0	1.02	0.3%	0.01%	1.02	0.3%	0.02%	1.01	0.3%	0.00%	**0.3%**	0.03%
	C22:1	1.01	0.3%	0.02%	1.01	0.3%	0.02%	1.01	0.4%	0.02%	**0.3%**	0.04%
	C24:0	1.05	0.1%	0.01%	1.05	0.1%	0.01%	1.21	0.1%	0.00%	**0.1%**	0.03%
	C24:1	1.05	0.1%	0.00%	1.05	0.1%	0.01%	1.21	0.2%	0.01%	**0.1%**	0.03%

a: correction factor

Table S- 2: Calculation of weight of individual FAMEs (FAME yield)

FAME Composition	TM-D, g/100 g seeds		TX-D, g/100 g seeds		TM+D, g/100 g seeds	
	mean	SD	mean	SD	mean	SD
C14:0	0.03	0.001	0.02	0.000	0.03	0.000
C16:0	1.85	0.062	1.92	0.005	2.11	0.003
C16:1	0.12	0.004	0.10	0.000	0.15	0.000
C18:0	0.61	0.020	0.70	0.002	0.70	0.001
C18:1	22.24	0.738	23.86	0.065	25.98	0.039
C18:2	6.61	0.219	6.70	0.018	7.64	0.011
C18:3	3.61	0.120	3.48	0.010	4.17	0.006
C20:0	0.19	0.006	0.24	0.001	0.23	0.000
C20:1	0.47	0.016	0.53	0.001	0.55	0.001
C22:0	0.11	0.004	0.14	0.000	0.14	0.000
C22:1	0.13	0.004	0.17	0.000	0.16	0.000
C24:0	0.04	0.001	0.07	0.000	0.05	0.000
C24:1	0.05	0.002	0.08	0.000	0.06	0.000
sum	36.07	1.197	38.01	0.104	41.98	0.062
	86%		91%		100%	

Calculation:

The internal standard C19:0 FAME with known amounts were added into each reactor for the determination of individual FAME yields. The yield of FAMEs in three transmethylation mixtures were calculated with the peak area ratio between C19:0 FAME and total FAMEs.

Table S- 3: Calculation of molecular weight of TAG of rapeseed oil

Fatty acid	MWi, Molar weight of FAi	fi, FA weight fraction	fi/MWi
C14:0	228.4	0.1%	3.5679E-06
C16:0	256.4	5.0%	1.9503E-04
C16:1	254.4	0.3%	1.3731E-05
C18:0	284.5	1.7%	5.9021E-05
C18:1	282.5	61.9%	2.1914E-03
C18:2	280.5	18.2%	6.4917E-04
C18:3	278.4	9.9%	3.5680E-04
C20:0	312.5	0.5%	1.7563E-05
C20:1	310.5	1.3%	4.2723E-05
C22:0	340.6	0.3%	9.5666E-06
C22:1	338.6	0.4%	1.1354E-05
C24:0	368.6	0.1%	3.3698E-06
C24:1	366.6	0.1%	3.7931E-06
Summe		1	3.5570E-03
average molecular weight of fatty acid mixture, g/mol		281.1	
Molar Weight of TAG in rapeseed oil, g/mol		881.4	

Calculation:

Average molecular weight of fatty acids = $\sum fi / \sum(fi/MWi)$; where fi is the FA weight fraction and MWi is the molar weight of FAi.

The molecular weight of the TAG: MW=3*Average molecular weight of fatty acids+38.049, where 3 means TAG contains three fatty acids and 38.049 is the weight of glycerol backbone.

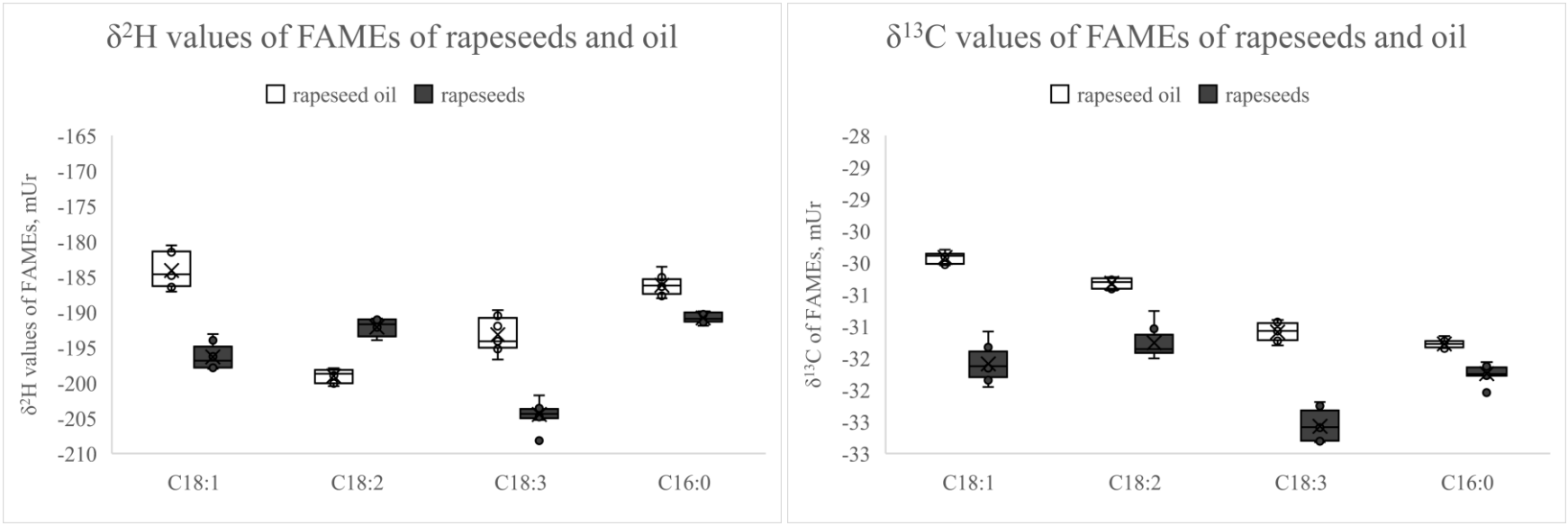

Figure S- 1: The results of carbon and hydrogen isotope compositions of the four major FAMEs of rapeseeds and rapeseed oil (C16:0, C18:1, C18:2 and C18:3)

Table S- 4: The $\delta^{13}C$ values of C16:0, C18:1, C18:2 and C18:3 FAME in rapeseed using transmethylation mixtures TX-D and TM+D (F-Test and T-Test)

transmethylation mixture		**$\delta^{13}C$ of C16:0 FAME**		**$\delta^{13}C$ of C18:1 FAME**		**$\delta^{13}C$ of C18:2 FAME**		**$\delta^{13}C$ of C18:3 FAME**	
		mean, n=3	SD, n=3	mean, n=3	SD, n=3	mean, n=3	SD, n=3	mean, n=3	SD, n=3
TX-D	sample 1	-31.72	0.12	-31.09	0.07	-31.00	0.14	-31.59	0.21
	sample 2	-31.96	0.02	-30.71	0.20	-30.56	0.13	-31.26	0.11
	sample 3	-31.98	0.03	-30.29	0.04	-30.68	0.03	-31.50	0.20
	mean, n=3	-31.88	-	-30.70	-	-30.75	-	-31.45	-
	SD, n=3	-	0.14	-	0.40	-	0.23	-	0.17
TM+D	sample 4	-31.56	0.02	-30.60	0.02	-30.61	0.17	-31.58	0.08
	sample 5	-32.00	0.03	-30.45	0.05	-30.53	0.05	-31.75	0.10
	sample 6	-31.98	0.05	-30.27	0.06	-30.68	0.13	-31.78	0.08
	mean, n=3	-31.85	-	-30.44	-	-30.60	-	-31.70	-
	SD, n=3	-	0.25	-	0.16	-	0.08	-	0.11
p value of F-Test		0.49		0.29		0.20		0.57	
p value of T-Test		0.82		0.36		0.36		0.09	

Table S- 5: The δ^2H values of C16:0, C18:1, C18:2 and C18:3 FAME in rapeseed using transmethylation mixtures TX-D and TM+D (F-Test and T-Test)

transmethylation mixture		**δ^2H of C16:0 FAME**		**δ^2H of C18:1 FAME**		**δ^2H of C18:2 FAME**		**δ^2H of C18:3 FAME**	
		mean n=3	SD n=3	mean n=3	SD n=3	mean n=3	SD n=3	mean n=3	SD n=3
TX-D	sample 1	-188.16	2.35	-190.15	2.58	-191.04	2.26	-206.49	2.71
	sample 2	-191.78	1.08	-196.71	1.31	-193.49	0.96	-207.21	1.08
	sample 3	-190.12	1.09	-195.99	0.74	-193.54	0.94	-207.36	1.15
	mean, n=3	-190.02	-	-194.28	-	-192.69	-	-207.02	-
	SD, n=3	-	1.81	-	3.60	-	1.43	-	0.46
TM+D	sample 4	-189.52	0.07	-194.44	1.62	-192.39	1.21	-203.67	1.67
	sample 5	-189.69	0.83	-197.02	1.20	-192.61	1.56	-205.37	2.44
	sample 6	-188.70	0.54	-197.51	0.56	-191.37	0.66	-204.32	0.79
	mean, n=3	-189.30	-	-196.32	-	-192.13	-	-204.45	-
	SD, n=3	-	0.53	-	1.65	-	0.66	-	0.86
p value of F-Test		0.16		0.35		0.35		0.45	
p value of T-Test		0.55		0.42		0.57		0.01^a	

a: p value < 0.05

Table S- 6: Calculation of $\Delta\delta^2H$ of FAME between TM+D and TX-D of C16:0, C18:1 and C18:2

FAME	H number of FA	H number of FAME	$\Delta\delta^2H$ of FAME between TX-D and TM+D (measured)	$\Delta\delta^2H$ of -CH3 of methanol between TX-D and TM+D (estimated)
C18:3	29	32	2.6	27.7

Calculation:

The relationship of δ^2 values between FAs and FAMEs can be estimated using the following mass balance equation (6)

$(H_n + 3) \times \delta^2H_{FAME} = H_n \times \delta^2H_{FA} + 3 \times \delta^2H_{MeOH}$

$(C_n + 1) \times \delta^{13}C_{FAME} = Cn \times \delta^{13}C_{FA} + 1 \times \delta^{13}C_{MeOH}$ equation (6)

Where H_n and Cn is the number of H and C atoms in fatty acid; δ^2H_{FAME} and $\delta^{13}C_{FAME}$, δ^2H_{FA} and $\delta^{13}C_{FA}$, δ^2H_{MeOH} and $\delta^{13}C_{MeOH}$ are the hydrogen and carbon isotope composition of FAME, FA and methyl group of methanol.

Assuming that C18:3 FAME by the TX-D is generated from the initially added methanol whereas that of TM+D is generated from the mixture of initially added and the reaction intermediate methanol, then the difference of δ2H values (Δδ2H) of methanol ($\delta2H_{MeOH}$) between TM+D and TX-D is 28 mUr, derived based on the measured Δδ2H of FAME 18:3 of 2.6 mUr and the equation

TX-D: $(H_n + 3) \times \delta^2H_{FAME} = H_n \times \delta^2H_{FA} + 3 \times \delta^2H_{MeOH}$ equation (6-1)

TM+D: $(H_n + 3) \times \delta^2H_{FAME} = H_n \times \delta^2H_{FA} + 3 \times \delta^2H_{MeOH}$ equation (6-2)

Equation (6-1) – equation (6-2):

δ^2H_{MeOH} in TX-D - δ^2H_{MeOH} in TM+D = $(H_n + 3) \times (\delta^2H_{FAME\ in\ TX\text{-}D} - \delta^2H_{FAME\ in\ TM+D})/3$ equation (6-3)

$\Delta\delta^2H_{MeOH}$ between TX-D and TM+D is 27.7 mUr, derived from the $\Delta\delta^2H_{FAME}$ between TX-D and TM+D of 2.6 mUr and equation (6-3).

FAME	H number of FA	H number of FAME	$\Delta\delta^2H$ of -CH3 of methanol between TX-D and TM+D	$\Delta\delta^2H$ of FAME between TX-D and TM+D
C16:0	31	34	27.7	2.4
C18:1	33	36	27.7	2.3
C18:2	31	34	27.7	2.4

Calculation:

$\delta^2H_{FAME\ in\ TX\text{-}D} - \delta^2H_{FAME\ in\ TM+D} = (\delta^2H_{MeOH}$ in TX-D - δ^2H_{MeOH} in TM+D) x 3/ $(H_n + 3)$ equation (6-4)

For the other FAMEs, the $\Delta\delta^2H_{FAME}$ between TX-D and TM+D should be about 2.3-2.4 mUr, derived based on $\Delta\delta^2H_{MeOH}$ between TX-D and TM+D of 28 mUr and equation (6-4).

Table S- 7 Estimation of $\delta^{13}C$ and $\delta^{2}H$ values of the mixed methanol in TM+D mixture

$\delta^{13}C$ value of methoxy group of DMP	$\delta^{13}C$ value of originally added Methanol	$\delta^{13}C$ value of mixture Methanol
assumed	measured	estimated
$\delta^{13}C_{H3CO\text{-}DMP}$, mUr	$\delta^{13}C_{MeOH}$, mUr	$\delta^{13}C_{H3CO\text{-}DMP+MeOH}$, mUr
-80	-38.9	-42.2
-20		-37.4

$\delta^{2}H$ value of methoxy group of DMP	$\delta^{2}H$ value of originally added Methanol (bulk)*	$\delta^{2}H$ value of mixture Methanol
assumed	measured	estimated
$\delta^{2}H_{H3CO\text{-}DMP}$, mUr	$\delta^{2}H_{MeOH}$, mUr	$\delta^{2}H_{H3CO\text{-}DMP+MeOH}$, mUr
-300	-169.5 mUr	-179.9
-20		-157.5

*The $\delta^{2}H$ value used for the calculation should be the $\delta^{2}H$ value of H_3CO- from methanol. It cannot be measured by GC-C/Py-IRMS.
So for the rough estimation, the bulk $\delta^{2}H$ value was used.

Calculation:

The $\delta^{13}C$ and $\delta^{2}H$ values of the mixed methanol, i.e. the originally added methanol + reaction intermediate methanol, in TM+D mixture was calculated using equation (7-1) and (7-2), respectively:

$$\delta^{13}C_{H3CO\text{-}DMP+MeOH} = a * \delta^{13}C_{H3CO\text{-}DMP} + b * \delta^{13}C_{MeOH} \quad \text{equation (7-1)}$$

$$\delta^{2}H_{H3CO\text{-}DMP+MeOH} = a * \delta^{2}H_{H3CO\text{-}DMP} + b * \delta^{2}H_{MeOH} \quad \text{equation (7-2)}$$

a: Faction of MeOH from DMP (0.08)

b: Faction of MeOH from MeOH (0.92)

Table S- 8: Estimation [i] of $\delta^{13}C_{FAME}$ in TM+D mixture and the difference ($\Delta\delta^{13}C_{FAME}$) between the measured and estimated values

$\delta^{13}C$ value of H_3CO-group of DMP	Comp.	number of C in FA	number of C in FAME	in TX-D, without DMP			in TM+D, with DMP			$\Delta\delta^{13}C_{FAME}$, mUr	
				$\delta^{13}C$ value of FAMEs	$\delta^{13}C$ value of methanol	$\delta^{13}C$ value of FAs	$\delta^{13}C$ value of FAMEs	$\delta^{13}C$ value of methanol	$\delta^{13}C$ value of FAMEs		
assumed				measured (a)	measured (b)	estimated (c)	measured (d)	estimated (e)	estimated (f)	A: (f)-(a)	B: (f)-(d)
$\delta^{13}C_{H3CO\text{-}DMP}$, mUr				$\delta^{13}C_{FAME}$, mUr	$\delta^{13}C_{MeOH}$, mUr	$\delta^{13}C_{FA}$, mUr	$\delta^{13}C_{FAME}$, mUr	$\delta^{13}C_{MeOH}$*, mUr	$\delta^{13}C_{FAME}$, mUr		
-80	C16:0	16	17	-31.88	-38.9	-31.4	-31.9	-42.2	-32.1	-0.2	-0.2
	C18:1	18	19	-30.7	-38.9	-30.2	-30.4	-42.2	-30.9	-0.2	-0.4
	C18:2	18	19	-30.75	-38.9	-30.3	-30.6	-42.2	-30.9	-0.2	-0.3
	C18:3	18	19	-31.45	-38.9	-31.0	-31.7	-42.2	-31.6	-0.2	0.1
-20	C16:0	16	17	-31.88	-38.9	-31.4	-31.9	-37.4	-31.8	0.1	0.1
	C18:1	18	19	-30.7	-38.9	-30.2	-30.4	-37.4	-30.6	0.1	-0.2
	C18:2	18	19	-30.75	-38.9	-30.3	-30.6	-37.4	-30.7	0.1	-0.1
	C18:3	18	19	-31.45	-38.9	-31.0	-31.7	-37.4	-31.4	0.1	0.3

[i] Estimation was made with an assumption that $\delta^{13}C_{H3CO\text{-}DMP}$ ranges from -80 to -20 mUr

(c): calculated based on the (a) and (b) values and the mass balance equation (6) in Supporting Information

(e): calculated based on the equation (7) in Supporting Information

(f): calculated based on the (d) and (e) values and the mass balance equation (6) in Supporting Information

∗ mixture of originally added methanol and the reaction intermediate methanol

Table S- 9: Estimation [i] of δ^2H_{FAME} in TM+D mixture and the difference ($\Delta\delta^2H_{FAME}$) between the measured and estimated values

δ^2H value of H_3CO-group of DMP	Comp.	number of H in FA	number of H in FAME	in TX-D, without DMP			in TM+D, with DMP			$\Delta\delta^2H_{FAME}$, mUr	
				δ^2H value of FAMEs	δ^2H value of methanol	δ^2H value of FAs	δ^2H value of FAMEs	δ^2H value of methanol	δ^2H value of FAMEs		
assumed				measured (a)	measured (b)	estimated (c)	measured (d)	estimated (e)	estimated (f)	A: (f)-(a)	B: (f)-(d)
$\delta^2H_{H3CO-DMP}$, mUr				δ^2H_{FAME}, mUr	δ^2H_{MeOH}, mUr	δ^2H_{FA}, mUr	δ^2H_{FAME}, mUr	δ^2H_{MeOH}*, mUr	δ^2H_{FAME}, mUr		
-300	C16:0	31	34	-190.0	-169.5	-192.0	-189.30	-179.9	-190.9	-0.9	-1.6
	C18:1	33	36	-194.3	-169.5	-196.6	-196.32	-179.9	-195.2	-0.9	1.2
	C18:2	31	34	-192.7	-169.5	-194.9	-192.13	-179.9	-193.6	-0.9	-1.5
	C18:3	29	32	-207.0	-169.5	-210.9	-204.45	-179.9	-208.0	-1.0	-3.5
-20	C16:0	31	34	-190.0	-169.5	-192.0	-189.30	-157.5	-188.9	1.1	0.4
	C18:1	33	36	-194.3	-169.5	-196.6	-196.32	-157.5	-193.3	1.0	3.0
	C18:2	31	34	-192.7	-169.5	-194.9	-192.13	-157.5	-191.6	1.1	0.5
	C18:3	29	32	-207.0	-169.5	-210.9	-204.45	-157.5	-205.9	1.1	-1.4

[i] Estimation was made with an assumption that $\delta^2H_{H3CO-DMP}$ ranges from -300 to -20 mUr.

(c): calculated based on the (a) and (b) values and the mass balance equation (6) in Supporting Information

(e): calculated based on the equation (7) in Supporting Information

(f): calculated based on the (d) and (e) values and the mass balance equation (6) in Supporting Information

* mixture of originally added methanol and the reaction intermediate methanol