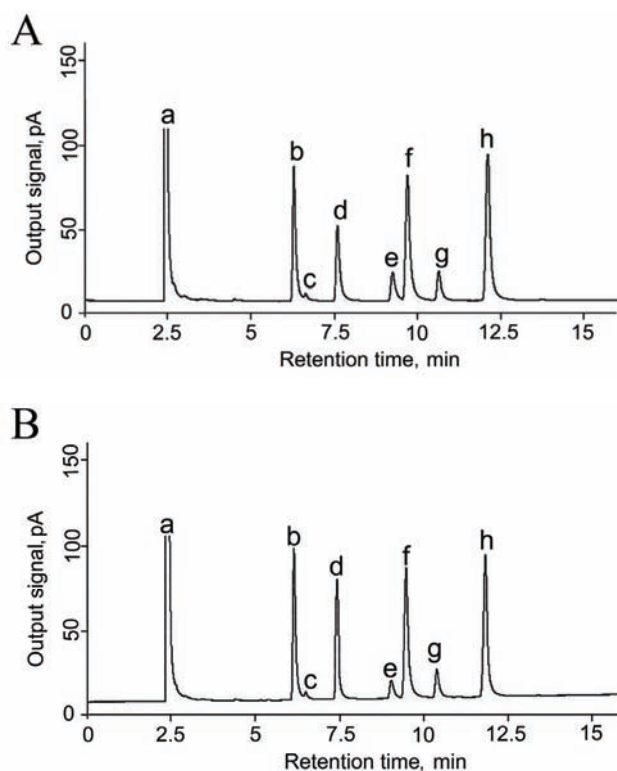


SUPPLEMENTARY MATERIAL TO
**An effective GC method for the determination of the fatty acid
composition in silkworm pupae oil using a two-step
methylation process**

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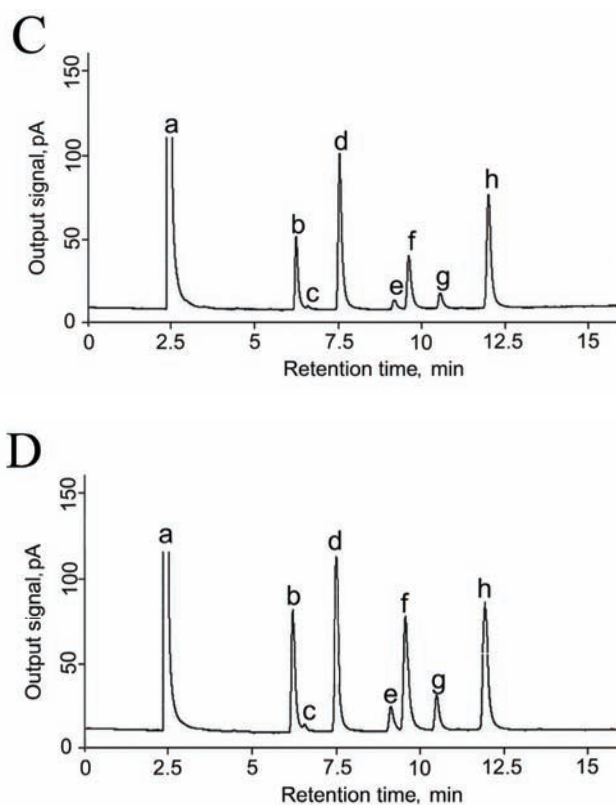


Fig. S-1. GC chromatograms of FAMES in pupae oil from silkworm variety 5078 prepared by different one-step methylation methods. A, KOH–MeOH methylation; B, H₂SO₄–MeOH methylation; C, BF₃–MeOH methylation; D, NaOCH₃–MeOH methylation. (a, *n*-hexane; b, methyl palmitate; c, methyl palmitoleate; d, methyl heptadecanoate; e, methyl stearate; f, methyl oleate; g, methyl linoleate; h, methyl linolenate).

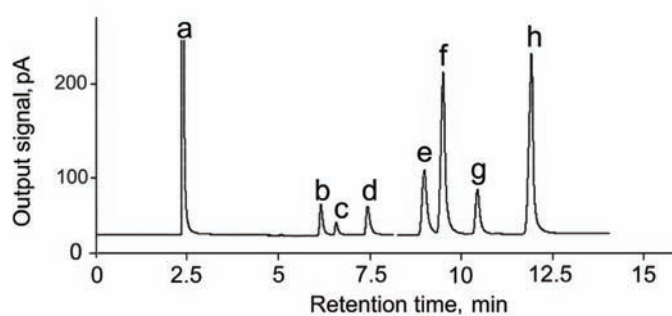
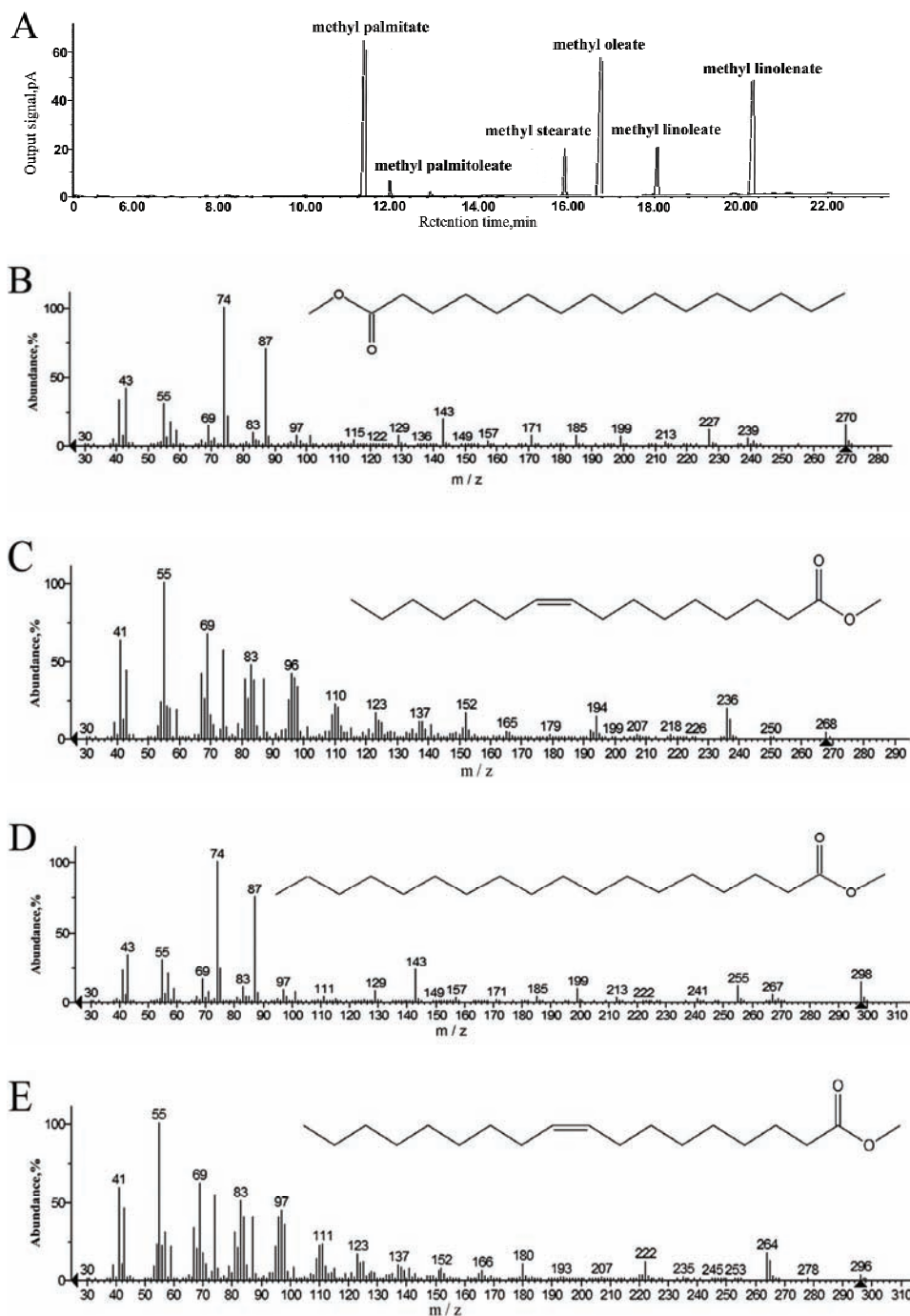


Fig. S-2. GC chromatogram of the six FAME standards (a, *n*-hexane; b, methyl palmitate; c, methyl palmitoleate; d, methyl heptadecanoate; e, methyl stearate; f, methyl oleate; g, methyl linoleate; h, methyl linolenate.)



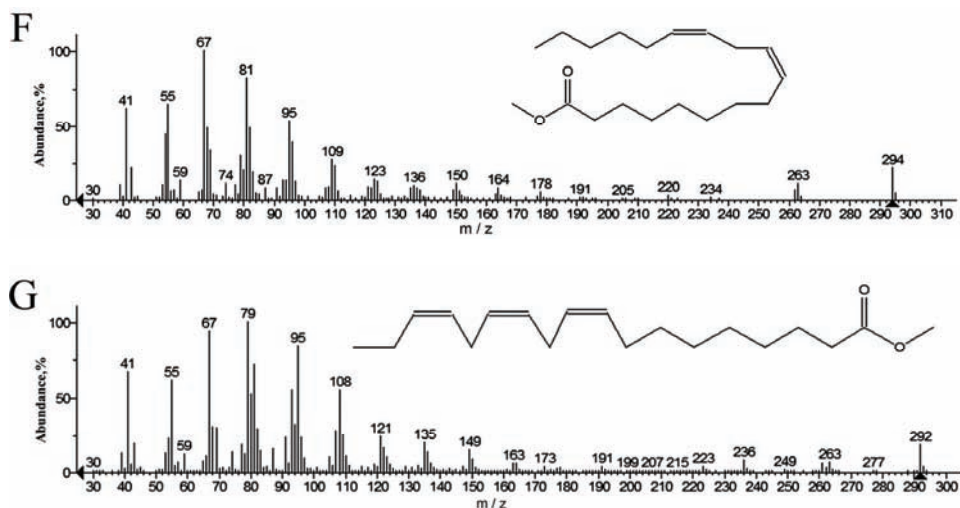


Fig. S-3. TIC chromatogram and mass spectra of FAMES in silkworm pupae oil using GC-MS. A, GC-MS confirmation of FAMES in silkworm pupae oil; B, mass spectrum of methyl palmitate, C, mass spectrum of methyl palmitoleate; D, mass spectrum of methyl stearate; E, mass spectrum of methyl oleate; F, mass spectrum of methyl linoleate; G, mass spectrum of methyl linolenate (m/z range from 35 to 450).

TABLE S-I. Linear regression equations of the FAME standard curves

Standard sample	Retention time, min	Linear range mg mL ⁻¹	Regression equation	R ²
Methyl palmitate	6.3	1.00–10.00	$y = 1.0167x - 0.0944$	0.9996
Methyl palmitoleate	6.6	0.05–0.50	$y = 0.8274x - 0.0033$	0.9999
Methyl stearate	9.2	0.25–2.50	$y = 1.4751x - 0.1339$	0.9999
Methyl oleate	9.6	1.00–10.00	$y = 0.9345x - 0.2010$	0.9998
Methyl linoleate	10.6	0.50–2.50	$y = 1.0387x - 0.3547$	0.9998
Methyl linolenate	12.1	1.00–10.00	$y = 0.8789x - 0.1959$	0.9998

TABLE S-II. Precision test results of FAME content of silkworm pupae oil obtained using five methylation methods

Fatty acid methyl ester	Method	Average value, mg mL ⁻¹	RSD / %	SE	<i>t</i> -Value	<i>p</i> -Value
Methyl palmitate (C16:0)	KOH	0.822	3.15	0.0614	13.39	0.0055
	H ₂ SO ₄	1.221	2.76	0.0239	51.03	0.0004
	BF ₃	0.626	8.76	0.0388	16.15	0.0038
	NaOCH ₃	0.517	4.23	0.0126	40.92	0.0006
	Two-step	1.425	5.82	0.0587	24.29	0.0017

TABLE S-II. Continued

Fatty acid methyl ester	Method	Average value, mg mL ⁻¹	RSD / %	SE	t-Value	p-Value
Methyl palmitoleate (C16:1)	KOH	0.060	1.02	0.0003	172.63	0.0001
	H ₂ SO ₄	0.099	2.22	0.0013	78.10	0.0002
	BF ₃	0.054	8.62	0.0033	16.41	0.0037
	NaOCH ₃	0.039	8.36	0.0019	20.63	0.0023
	Two-step	0.128	4.37	0.0039	32.40	0.0010
Methyl stearate (C18:0)	KOH	0.234	10.64	0.0176	13.30	0.0056
	H ₂ SO ₄	0.324	1.07	0.0024	132.45	0.0001
	BF ₃	0.189	5.66	0.0076	25.01	0.0016
	NaOCH ₃	0.185	3.11	0.0033	55.86	0.0003
Methyl oleate (C18:1)	Two-step	0.370	4.97	0.0130	28.46	0.0012
	KOH	1.220	9.52	0.1304	3.35	0.0112
	H ₂ SO ₄	1.827	1.67	0.0216	84.46	0.0001
	BF ₃	0.918	2.20	0.0586	15.67	0.0040
	NaOCH ₃	0.835	4.65	0.0224	37.26	0.0007
Methyl linoleate (C18:2)	Two-step	2.227	2.39	0.0968	23.00	0.0019
	KOH	0.520	3.82	0.0219	23.80	0.0018
	H ₂ SO ₄	0.651	0.98	0.0045	144.49	0.0001
	BF ₃	0.506	2.95	0.0105	48.04	0.0004
	NaOCH ₃	0.522	1.26	0.0720	7.24	0.0186
Methyl linolenate (C18:3)	Two-step	0.741	4.44	0.0233	31.84	0.0010
	KOH	1.453	10.30	0.1542	9.43	0.0111
	H ₂ SO ₄	2.288	2.28	0.0368	62.10	0.0003
	BF ₃	1.375	3.35	0.0872	15.74	0.0040
	NaOCH ₃	0.976	4.79	0.0270	36.20	0.0008
	Two-step	2.756	3.05	0.1153	23.91	0.0017

TABLE S-III. FAME content (mg mL⁻¹) of silkworm pupae oil after a week obtained using five methylation methods

Method	Methyl palmitate	Methyl palmitoleate	Methyl stearate	Methyl oleate	Methyl linoleate	Methyl linolenate
KOH	1.313±	0.117±	0.330±	2.016±	0.667±	2.380±
	0.013	0.001	0.004	0.096	0.001	0.002
H ₂ SO ₄	1.302±	0.120±	0.333±	1.941±	0.674±	2.469±
	0.005	0.008	0.007	0.014	0.001	0.026
BF ₃	0.871±	0.074±	0.235±	1.295±	0.597±	1.884±
	0.015	0.001	0.003	0.002	0.011	0.022
NaOCH ₃	1.485±	0.126±	0.375±	2.288±	0.723±	2.792±
	0.027	0.018	0.005	0.042	0.011	0.072
Two-step	1.909±	0.165±	0.449±	2.891±	0.863±	3.614±
	0.009	0.012	0.015	0.049	0.020	0.010

TABLE S-IV. Average recovery results of FAMES of silkworm pupae oil obtained using five methylation methods

Method	Fatty acid methyl ester	Average recovery, %	RSD / %
KOH	Methyl palmitate (C16:0)	120.47	0.71
	Methyl palmitoleate (C16:1)	80.62	17.17
	Methyl stearate (C18:0)	119.96	4.82
	Methyl oleate (C18:1)	89.36	8.00
	Methyl linoleate (C18:2)	114.84	3.38
	Methyl linolenate (C18:3)	77.78	0.67
H ₂ SO ₄	Methyl palmitate (C16:0)	119.02	5.13
	Methyl palmitoleate (C16:1)	116.01	9.72
	Methyl stearate (C18:0)	119.96	1.57
	Methyl oleate (C18:1)	108.77	3.19
	Methyl linoleate (C18:2)	123.31	0.48
	Methyl linolenate (C18:3)	84.43	5.59
BF ₃	Methyl palmitate (C16:0)	118.99	2.37
	Methyl palmitoleate (C16:1)	102.95	16.36
	Methyl stearate (C18:0)	111.54	19.50
	Methyl oleate (C18:1)	103.15	11.86
	Methyl linoleate (C18:2)	116.25	4.61
	Methyl linolenate (C18:3)	77.78	6.09
NaOCH ₃	Methyl palmitate (C16:0)	114.34	1.62
	Methyl palmitoleate (C16:1)	103.86	1.33
	Methyl stearate (C18:0)	100.91	3.62
	Methyl oleate (C18:1)	101.74	8.17
	Methyl linoleate (C18:2)	115.68	4.66
	Methyl linolenate (C18:3)	76.13	7.22
Two-step	Methyl palmitate (C16:0)	120.66	0.47
	Methyl palmitoleate (C16:1)	112.61	3.24
	Methyl stearate (C18:0)	113.64	2.57
	Methyl oleate (C18:1)	109.66	3.06
	Methyl linoleate (C18:2)	114.40	0.40
	Methyl linolenate (C18:3)	85.02	4.47