



AOCS Official Method Ce 2b-11

Revised 2013

Direct Methylation of Lipids in Foods by Alkali Hydrolysis

DEFINITION

This method describes a simultaneous alkali hydrolysis and methylation procedure without prior digestion option for the preparation of fatty acid methyl esters (FAMES) directly from food matrices. The incorporation of triacylglycerol (TAG) standards allows the quantification of total fat and fatty acids using Theoretical Correction Factors (TCFs) and Empirical Correction Factors (ECFs).

SCOPE

This method may be applicable for fat-containing matrices (e.g., food stuffs, beverages, tissues, and oils). The results of the collaborative study indicate that most matrices do not require acid pretreatment. Extruded cat, dog foods and oat based foods gave low results, thus, analysts are advised to test the chosen method against appropriate reference methods. Materials with high moisture contents do not require drying before analysis. However, if drying is required (except when freeze drying) antioxidants should be added to protect from oxidation. An appropriate grinding technique for homogenization of the test sample should be used, when necessary.

This method is not suitable for steryl ester concentrates and steryl ester-fortified food products.

APPARATUS

1. 50-mL round flat-bottom flasks with 24/40 joints or equivalent
2. Teflon™ flask-neck sleeves (size 24/40)
3. Hollow, full-length stopper (size 24/40), barrel-shaped head
4. Explosion-proof hot plates or equivalent (see Notes, 1)
5. Water-cooled condensers or equivalent (see Notes, 1)
6. 2-mL amber write-on autosampler vials with Teflon™-lined caps
7. Glass powder funnel
8. Analytical balance capable of reading to ± 0.0001 g or ± 0.1 mg
9. Weighing funnels
10. 1-L transparent polymethylpentene screw top flask
11. 500-mL Nalgene® bottle
12. Porous boiling chips

REAGENTS

1. Sodium Hydroxide, reagent grade
2. Methanol (MeOH), high purity gas chromatographic grade
3. NaOH/MeOH (0.5 M NaOH in MeOH). Add 20 g of NaOH to a 1-L polymethylpentene flask. Add MeOH and mix well to dissolve before diluting to volume.
4. Sodium Chloride, reagent grade, saturated. Dissolve 36 g NaCl in 100 mL distilled water.
5. Hexanes or *n*-heptane, high purity gas chromatographic grade
6. Chloroform, high purity gas chromatographic grade
7. 14% Boron Trifluoride in MeOH (see Notes, 2)
8. Sodium Sulfate, anhydrous, reagent grade
9. Pyrogallol, ACS reagent
10. Internal standard: 13:0, Tritridecanoin (P/N T-135, Nu-Chek-Prep, Inc. or equivalent) for unknowns, dairy or ruminant fats. Accurately weigh out 2.5 g of 13:0 triacylglycerol (TAG) into a weighing funnel. Empty the weighing funnel into a 500-mL class A volumetric flask and rinse the funnel with hexanes or *n*-heptane to ensure complete quantitative transfer into the flask. Add hexanes or *n*-heptane to the flask, mix to dissolve, and dilute to volume (see Calculations, 1). The 13:0 TAG internal standard solution is stable indefinitely if precautions are taken to eliminate the loss of *n*-heptane and therefore a change in the concentration of the internal standard.
11. Internal standard: 21:0, Triheneicosanoin (P/N T-175, Nu-Chek-Prep, Inc. or equivalent) for all samples other than dairy or ruminant fats and marine or long chain polyunsaturated oils. Accurately weigh out 2.5 g of 21:0 TAG into a weighing funnel. Empty the weighing funnel into a 500-mL class A volumetric flask and rinse the funnel with chloroform to ensure complete quantitative transfer into the flask. Add chloroform to the flask, mix to dissolve, and dilute to volume (see Calculations,

Table 1
Guide to test portion size and internal standard volume.

Expected Fat or Oil Concentration	Test Portion Amount (mg)	Fat or Oil Concentration (mg/mL)	Fat or Oil Amount (mg)	Amount of TAG IS Added (mg)	13:0 TAG IS (mL)	21:0 TAG IS (mL)	23:0 TAG IS (mL)
90–100%	100	18–20	90–100	4.5–10	1–2	1–2	2–5
80–89%	100	16–18	80–90	4–9	1–2	1–2	2–5
70–79%	125	17.5–20	87.5–100	4.4–10	1–2	1–2	2–5
60–69%	125	15–17.5	75–87.5	3.8–8.8	1–2	1–2	2–5
50–59%	150	15–18	75–90	3.8–9	1–2	1–2	2–5
40–49%	200	16–20	80–100	4–10	1–2	1–2	2–5
30–39%	250	15–20	75–100	3.8–10	1–2	1–2	2–5
20–29%	325	13–19.5	65–97.5	3.3–9.8	1–2	1–2	2–5
10–19%	500	10–20	50–100	2.5–10	0.5–2	0.5–2	1–5
ND–9%	1000	<20	<100	<10	0.5–2	0.5–2	1–5

Table 2
Results of *cis*-, *trans*-, Saturated, Monounsaturated, and Polyunsaturated fatty acid determination by 9 laboratories in a collaborative study organized by AOCS in 2011. FAMES were analyzed using AOCS Ce 1j-07.

Anhydrous milk fat	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	93.59	88.92	58.27	5.11	26.39	21.59	3.78	3.25	0.53	2.64
Repeatability (Sr)	2.48	2.39	1.32	0.41	1.21	0.27	0.39	0.20	0.06	0.04
%RSDr	2.65	2.69	2.26	8.06	4.60	1.27	10.23	6.08	11.21	1.60
r (2.8xSr)	6.94	6.69	3.69	1.15	3.40	0.77	1.08	0.55	0.17	0.12
Reproducibility (SR)	2.48	2.39	2.39	0.67	1.53	0.73	0.54	0.20	0.14	0.24
%RSDR	2.65	2.69	4.10	13.14	5.81	3.38	14.28	6.08	26.83	8.94
R (2.8xSR)	6.94	6.69	6.69	1.88	4.29	2.05	1.51	0.55	0.40	0.66
Tallow	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	99.68	95.21	45.44	7.14	46.53	39.31	2.65	1.99	0.33	1.66
Repeatability (Sr)	1.01	0.96	1.03	0.19	0.78	0.33	0.12	0.05	0.01	0.04
%RSDr	1.01	1.01	2.26	2.73	1.67	0.84	4.43	2.41	3.80	2.33
r (2.8xSr)	2.82	2.69	2.88	0.55	2.18	0.92	0.33	0.13	0.04	0.11
Reproducibility (SR)	1.66	1.58	1.36	0.30	1.71	0.80	0.34	0.24	0.07	0.18
%RSDR	1.66	1.66	2.99	4.20	3.68	2.03	12.74	12.24	22.64	11.13
R (2.8xSR)	4.64	4.42	3.81	0.84	4.80	2.24	0.94	0.68	0.21	0.52
Chocolate cake mix	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	10.81	10.34	4.44	0.90	3.21	2.47	2.65	2.49	0.23	2.27
Repeatability (Sr)	0.23	0.22	0.11	0.03	0.06	0.04	0.07	0.06	0.01	0.05
%RSDr	2.09	2.09	2.37	3.29	1.79	1.72	2.77	2.60	2.46	2.20
r (2.8xSr)	0.63	0.61	0.30	0.08	0.16	0.12	0.21	0.18	0.02	0.14
Reproducibility (SR)	0.42	0.40	0.25	0.07	0.10	0.09	0.12	0.10	0.01	0.08
%RSDR	3.92	3.91	5.68	7.43	3.25	3.45	4.67	4.20	4.63	3.62
R (2.8xSR)	1.19	1.13	0.71	0.19	0.29	0.24	0.35	0.29	0.03	0.23

Table 2
(Continued)

Cheese Powder	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	29.78	28.38	13.49	7.27	14.08	6.95	1.24	0.97	0.14	0.83
Repeatability (Sr)	0.43	0.41	0.22	0.12	0.22	0.12	0.04	0.02	0.00	0.01
%RSDr	1.43	1.44	1.62	1.68	1.55	1.66	3.10	2.22	3.22	1.60
r (2.8xSr)	1.19	1.14	0.61	0.34	0.61	0.32	0.11	0.06	0.01	0.04
Reproducibility (SR)	2.39	2.28	0.71	0.37	0.75	0.57	0.12	0.02	0.03	0.05
%RSDR	8.02	8.02	5.24	5.04	5.31	8.18	9.54	2.46	19.48	6.58
R (2.8xSR)	6.69	6.38	1.98	1.03	2.09	1.59	0.33	0.07	0.08	0.15

DHA/EPA fortified infant formula	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	28.94	27.58	12.07	0.15	10.15	10.07	5.21	5.14	0.53	4.62
Repeatability (Sr)	0.66	0.63	0.07	0.02	0.20	0.20	0.13	0.12	0.01	0.09
%RSDr	2.29	2.29	0.59	15.01	1.96	2.02	2.43	2.41	2.16	2.01
r (2.8xSr)	1.86	1.77	0.20	0.06	0.56	0.57	0.35	0.35	0.03	0.26
Reproducibility (SR)	1.48	1.42	0.45	0.12	0.65	0.65	0.39	0.38	0.02	0.33
%RSDR	5.11	5.14	3.77	78.47	6.39	6.46	7.52	7.48	3.49	7.08
R (2.8xSR)	4.14	3.97	1.27	0.33	1.82	1.82	1.10	1.08	0.05	0.92

Extruded dog food (high protein and high fat)	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	22.04	21.06	6.72	0.31	9.75	9.31	4.76	4.72	0.33	4.36
Repeatability (Sr)	1.49	1.42	0.43	0.11	0.64	0.61	0.31	0.30	0.03	0.28
%RSDr	6.75	6.75	6.38	34.60	6.53	6.60	6.44	6.41	9.24	6.41
r (2.8xSr)	4.17	3.98	1.20	0.30	1.78	1.72	0.86	0.85	0.09	0.78
Reproducibility (SR)	1.49	1.42	0.43	0.11	0.64	0.61	0.31	0.30	0.04	0.28
%RSDR	6.75	6.75	6.38	34.97	6.53	6.60	6.44	6.41	11.77	6.41
R (2.8xSR)	4.17	3.98	1.20	0.31	1.78	1.72	0.86	0.85	0.11	0.78

Oatmeal Cookie	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	19.19	18.33	8.95	0.05	7.12	7.08	2.25	2.20	0.05	2.16
Repeatability (Sr)	0.27	0.26	0.20	0.02	0.08	0.08	0.02	0.02	0.00	0.02
%RSDr	1.42	1.42	2.23	28.55	1.13	1.11	1.03	1.02	2.41	0.99
r (2.8xSr)	0.76	0.73	0.56	0.04	0.22	0.22	0.06	0.06	0.00	0.06
Reproducibility (SR)	0.72	0.68	0.39	0.02	0.31	0.31	0.09	0.10	0.00	0.09
%RSDR	3.74	3.72	4.30	44.84	4.38	4.40	4.00	4.33	4.89	4.36
R (2.8xSR)	2.01	1.91	1.08	0.07	0.87	0.87	0.25	0.27	0.01	0.26

Evaporated milk	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	6.28	5.97	3.93	0.33	1.78	1.47	0.23	0.19	0.03	0.16
Repeatability (Sr)	0.29	0.27	0.17	0.03	0.09	0.08	0.02	0.01	0.00	0.01
%RSDr	4.61	4.57	4.44	8.45	5.11	5.17	7.06	4.73	8.53	4.38
r (2.8xSr)	0.81	0.76	0.49	0.08	0.25	0.21	0.05	0.03	0.01	0.02
Reproducibility (SR)	0.29	0.27	0.22	0.05	0.09	0.08	0.03	0.02	0.01	0.02
%RSDR	4.61	4.57	5.54	15.89	5.11	5.17	14.13	10.75	32.47	10.66
R (2.8xSR)	0.81	0.76	0.61	0.15	0.25	0.21	0.09	0.06	0.03	0.05

Table 2
(Continued)

Peanut butter	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	54.01	51.69	10.11	0.06	30.28	30.13	11.23	11.17	0.08	10.79
Repeatability (Sr)	1.17	1.12	0.33	0.01	0.70	0.74	0.29	0.40	0.02	0.26
%RSDr	2.16	2.16	3.29	21.71	2.31	2.47	2.61	3.62	21.36	2.37
r (2.8xSr)	3.26	3.12	0.93	0.03	1.96	2.08	0.82	1.13	0.05	0.72
Reproducibility (SR)	3.10	2.96	0.79	0.04	1.57	1.60	1.46	1.48	0.08	1.14
%RSDR	5.73	5.73	7.83	75.73	5.17	5.32	13.02	13.21	98.99	10.58
R (2.8xSR)	8.67	8.30	2.22	0.12	4.38	4.49	4.10	4.13	0.22	3.19

Yogurt (plain)	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	5.80	5.51	3.60	0.32	1.65	1.37	0.23	0.19	0.03	0.15
Repeatability (Sr)	0.55	0.53	0.39	0.03	0.13	0.11	0.03	0.02	0.01	0.01
%RSDr	9.54	9.55	10.90	7.94	8.09	8.26	12.67	13.02	42.60	9.08
r (2.8xSr)	1.55	1.47	1.10	0.07	0.37	0.32	0.08	0.07	0.04	0.04
Reproducibility (SR)	0.55	0.53	0.39	0.03	0.15	0.14	0.03	0.02	0.02	0.01
%RSDR	9.54	9.55	10.90	7.94	9.23	9.92	12.83	13.02	59.74	9.08
R (2.8xSR)	1.55	1.47	1.10	0.07	0.43	0.38	0.08	0.07	0.05	0.04

Canned cat food	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	5.70	5.44	1.71	0.05	2.40	2.01	1.25	1.24	0.42	0.76
Repeatability (Sr)	0.66	0.64	0.15	0.01	0.25	0.23	0.12	0.11	0.05	0.07
%RSDr	11.59	11.67	8.83	13.55	10.58	11.68	9.22	9.19	11.58	9.53
r (2.8xSr)	1.85	1.78	0.42	0.02	0.71	0.66	0.32	0.32	0.14	0.20
Reproducibility (SR)	0.80	0.77	0.26	0.03	0.46	0.93	0.23	0.22	0.24	0.14
%RSDR	14.09	14.11	15.08	49.55	19.14	46.30	18.07	17.88	56.88	18.16
R (2.8xSR)	2.25	2.15	0.72	0.08	1.29	2.60	0.63	0.62	0.68	0.39

DHA/EPA fortified orange juice	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	0.12	0.11	0.03	0.00	0.03	0.03	0.03	0.03	0.02	0.01
Repeatability (Sr)	0.02	0.02	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01
%RSDr	15.70	17.14	13.26	0.00	8.92	16.09	29.64	29.64	25.75	46.97
r (2.8xSr)	0.05	0.05	0.01	0.00	0.01	0.01	0.03	0.03	0.01	0.02
Reproducibility (SR)	0.02	0.02	0.02	0.00	0.00	0.01	0.02	0.02	0.01	0.01
%RSDR	19.39	20.56	51.60	0.00	12.41	19.13	58.12	58.12	67.03	58.62
R (2.8xSR)	0.06	0.06	0.05	0.00	0.01	0.01	0.05	0.05	0.03	0.02

Butter	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	71.12	67.76	36.52	2.49	22.31	20.23	8.77	8.36	1.73	6.91
Repeatability (Sr)	1.62	1.54	1.00	0.13	0.63	0.54	0.44	0.39	0.14	0.14
%RSDr	2.28	2.28	2.73	5.34	2.81	2.68	4.99	4.72	8.25	2.05
r (2.8xSr)	4.54	4.32	2.80	0.37	1.75	1.52	1.23	1.10	0.40	0.40
Reproducibility (SR)	4.74	4.52	2.32	0.43	1.94	1.74	0.89	0.88	0.15	0.50
%RSDR	6.66	6.68	6.35	17.29	8.69	8.62	10.15	10.50	8.89	7.17
R (2.8xSR)	13.26	12.66	6.49	1.21	5.43	4.88	2.49	2.46	0.43	1.39

Table 2
(Continued)

Whole egg powder	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	40.24	38.47	14.34	0.43	16.73	16.35	7.36	7.31	0.37	6.84
Repeatability (Sr)	1.20	1.15	0.41	0.02	0.56	0.56	0.22	0.21	0.02	0.20
%RSDr	2.98	2.98	2.85	4.79	3.33	3.41	2.95	2.92	5.99	2.88
r (2.8xSr)	3.36	3.21	1.14	0.06	1.56	1.56	0.61	0.60	0.06	0.55
Reproducibility (SR)	1.20	1.15	0.50	0.06	0.56	0.56	0.35	0.33	0.03	0.38
%RSDR	2.98	2.98	3.52	12.99	3.33	3.41	4.79	4.50	9.04	5.56
R (2.8xSR)	3.36	3.21	1.41	0.16	1.56	1.56	0.99	0.92	0.09	1.06
Full-fat soy flour flakes	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	23.05	22.05	3.70	0.02	4.73	4.74	13.49	13.48	1.61	11.69
Repeatability (Sr)	0.23	0.22	0.11	0.00	0.12	0.05	0.39	0.39	0.03	0.12
%RSDr	0.98	0.98	3.02	22.12	2.55	1.10	2.91	2.91	1.61	1.07
r (2.8xSr)	0.63	0.61	0.31	0.01	0.34	0.15	1.10	1.10	0.07	0.35
Reproducibility (SR)	0.66	0.62	0.39	0.01	0.14	0.12	0.39	0.39	0.15	0.25
%RSDR	2.85	2.83	10.42	73.10	2.93	2.57	2.91	2.91	9.34	2.13
R (2.8xSR)	1.84	1.75	1.08	0.04	0.39	0.34	1.10	1.10	0.42	0.70
Encapsulated DHA/EPA	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	56.05	53.66	18.18	0.68	14.09	13.33	21.02	20.63	18.25	2.19
Repeatability (Sr)	1.31	1.26	0.43	0.08	0.37	0.35	0.47	0.46	0.41	0.09
%RSDr	2.34	2.34	2.36	12.13	2.65	2.64	2.23	2.21	2.25	4.24
r (2.8xSr)	3.68	3.52	1.20	0.23	1.05	0.98	1.31	1.28	1.15	0.26
Reproducibility (SR)	3.32	3.15	0.95	0.23	0.81	0.67	1.83	1.75	1.46	0.93
%RSDR	5.92	5.87	5.22	33.82	5.72	5.01	8.71	8.50	8.01	42.40
R (2.8xSR)	9.29	8.83	2.66	0.64	2.26	1.87	5.13	4.91	4.09	2.60
Creamy ranch dressing	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	46.18	44.16	7.12	0.24	9.66	9.63	25.68	25.51	3.40	23.18
Repeatability (Sr)	1.91	1.82	0.32	0.01	0.52	0.52	0.98	0.98	0.11	0.92
%RSDr	4.13	4.13	4.51	3.71	5.39	5.40	3.83	3.84	3.18	3.98
r (2.8xSr)	5.34	5.11	0.90	0.02	1.46	1.46	2.75	2.74	0.30	2.58
Reproducibility (SR)	1.91	1.82	0.32	0.16	0.52	0.52	2.36	2.37	0.11	1.16
%RSDR	4.13	4.13	4.51	65.50	5.39	5.40	9.17	9.30	3.18	5.02
R (2.8xSR)	5.34	5.11	0.90	0.44	1.46	1.46	6.60	6.64	0.30	3.26
Potato chips	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	36.01	34.44	6.35	0.22	14.16	14.02	13.70	13.59	0.12	13.45
Repeatability (Sr)	1.75	1.67	0.31	0.06	0.74	0.72	0.63	0.61	0.02	0.60
%RSDr	4.85	4.85	4.95	26.22	5.21	5.14	4.57	4.50	13.30	4.50
r (2.8xSr)	4.89	4.68	0.88	0.16	2.07	2.02	1.75	1.71	0.04	1.69
Reproducibility (SR)	1.75	1.67	0.31	0.14	0.74	0.72	0.63	0.61	0.04	0.60
%RSDR	4.85	4.85	4.95	62.69	5.21	5.14	4.57	4.50	37.66	4.50
R (2.8xSR)	4.89	4.68	0.88	0.39	2.07	2.02	1.75	1.71	0.12	1.69

Table 2
(Continued)

Cheese powder duplicate	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	30.10	28.69	13.35	7.20	13.85	6.96	1.31	0.98	0.13	0.85
Repeatability (Sr)	0.45	0.43	0.27	0.10	0.22	0.11	0.05	0.02	0.01	0.02
%RSDr	1.49	1.48	2.05	1.36	1.59	1.56	4.14	2.12	5.11	2.18
r (2.8xSr)	1.25	1.19	0.77	0.27	0.62	0.30	0.15	0.06	0.02	0.05
Reproducibility (SR)	1.18	1.11	0.30	0.31	0.37	0.21	0.05	0.10	0.04	0.08
%RSDR	3.91	3.88	2.22	4.27	2.70	3.04	4.14	10.08	29.05	9.25
R (2.8xSR)	3.30	3.12	0.83	0.86	1.05	0.59	0.15	0.28	0.11	0.22
Frozen cheese pizza	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	8.06	7.66	3.56	0.37	2.25	1.68	1.33	1.29	0.05	1.23
Repeatability (Sr)	0.67	0.64	0.30	0.04	0.20	0.16	0.13	0.12	0.01	0.12
%RSDr	8.32	8.37	8.54	10.04	9.10	9.76	9.45	9.46	20.01	9.39
r (2.8xSr)	1.88	1.80	0.85	0.10	0.57	0.46	0.35	0.34	0.03	0.32
Reproducibility (SR)	1.59	1.51	1.63	0.07	0.45	0.77	0.24	0.24	0.03	0.23
%RSDR	19.72	19.76	45.76	18.70	19.92	45.85	18.19	18.38	56.05	18.41
R (2.8xSR)	4.45	4.24	4.56	0.19	1.25	2.15	0.68	0.66	0.07	0.64
Peanut butter duplicate	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	51.50	49.29	9.66	0.05	30.84	30.55	8.78	8.77	0.08	8.55
Repeatability (Sr)	3.97	3.79	0.88	0.03	2.40	2.26	0.59	0.59	0.01	0.59
%RSDr	7.70	7.70	9.13	53.93	7.79	7.41	6.76	6.77	6.55	6.91
r (2.8xSr)	11.10	10.62	2.47	0.08	6.73	6.34	1.66	1.66	0.02	1.65
Reproducibility (SR)	4.43	4.24	0.92	0.05	2.60	2.89	1.51	1.50	0.09	1.22
%RSDR	8.61	8.61	9.51	85.63	8.42	9.46	17.22	17.15	105.13	14.32
R (2.8xSR)	12.41	11.88	2.57	0.13	7.27	8.09	4.23	4.21	0.24	3.43
Dry cereal fortified with flax	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	1.91	1.82	0.28	0.00	0.36	0.36	1.20	1.20	0.52	0.64
Repeatability (Sr)	0.03	0.02	0.02	0.00	0.01	0.01	0.03	0.03	0.01	0.02
%RSDr	1.34	1.35	6.75	0.00	2.04	2.04	2.53	2.53	2.48	3.42
r (2.8xSr)	0.07	0.07	0.05	0.00	0.02	0.02	0.08	0.08	0.04	0.06
Reproducibility (SR)	0.27	0.26	0.04	0.00	0.06	0.06	0.09	0.09	0.03	0.09
%RSDR	14.17	14.19	14.49	0.00	16.81	16.81	7.26	7.26	6.12	13.26
R (2.8xSR)	0.76	0.72	0.12	0.00	0.17	0.17	0.24	0.24	0.09	0.24
Horse feed	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	3.22	3.07	0.72	0.00	0.76	0.75	1.58	1.58	0.21	1.38
Repeatability (Sr)	0.17	0.16	0.04	0.00	0.05	0.04	0.07	0.07	0.01	0.06
%RSDr	5.31	5.26	6.22	0.00	6.27	5.89	4.52	4.52	3.57	4.68
r (2.8xSr)	0.48	0.45	0.13	0.00	0.13	0.12	0.20	0.20	0.02	0.18
Reproducibility (SR)	0.19	0.18	0.07	0.00	0.06	0.04	0.09	0.09	0.02	0.07
%RSDR	5.86	5.82	9.52	0.00	7.85	5.89	5.54	5.54	8.39	5.36
R (2.8xSR)	0.53	0.50	0.19	0.00	0.17	0.12	0.25	0.25	0.05	0.21

Table 2
(Continued)

Gamebird feed	Total Fat (TAG%)*	Total Fat (FA%)*	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total <i>cis</i> PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	9	9	9	9	9	9	9
Mean	3.00	2.87	0.57	0.00	0.64	0.63	1.65	1.65	0.13	1.52
Repeatability (Sr)	0.07	0.07	0.02	0.00	0.02	0.02	0.02	0.02	0.00	0.02
%RSDr	2.25	2.29	4.27	0.00	3.40	3.47	1.49	1.44	1.68	1.50
r (2.8xSr)	0.19	0.18	0.07	0.00	0.06	0.06	0.07	0.07	0.01	0.06
Reproducibility (SR)	0.40	0.38	0.07	0.00	0.08	0.07	0.26	0.26	0.03	0.23
%RSDR	13.39	13.41	12.91	0.00	12.14	10.97	15.48	15.46	19.07	15.19
R (2.8xSR)	1.12	1.08	0.21	0.00	0.22	0.19	0.72	0.71	0.07	0.64

*NLEA (Nutritional Labeling and Education Act) according to the Office of Nutritional Products, Labeling, and Dietary Supplements in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration

**Does not include CLA

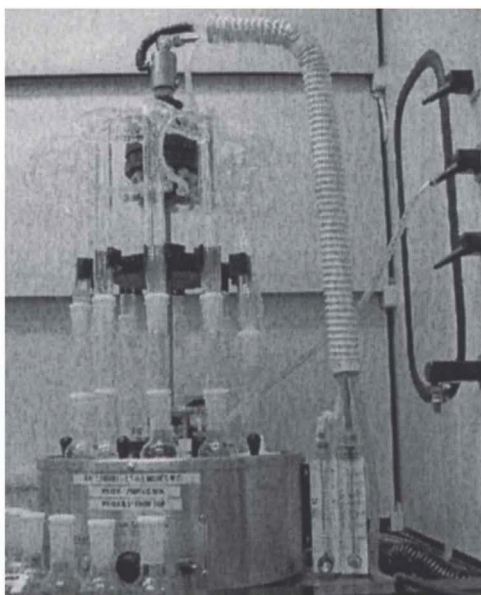


Figure 1. Example of suitable extraction apparatus (see Notes, 1)



AOCS Official Method Ce 2c-11

Revised 2013

Direct Methylation of Lipids in Foods by Acid-Alkali Hydrolysis

DEFINITION

This method describes an acid-alkaline procedure for the preparation of fatty acid methyl esters (FAMES) directly from food matrices. The fats or oils are released from the matrix by *in situ* acid digestion (hydrochloric acid in methanol) followed by alkali hydrolysis (sodium hydroxide in methanol) and methylation (with boron trifluoride in methanol as the catalyst).

SCOPE

This method is only required when AOCS Official Method Ce 2b-11 does not release all fatty acids quantitatively. Some food matrices such as extruded cat and dog foods, oat based foods, and some encapsulated oils require acid pretreatment. Some long chain polyunsaturated fortified beverages show lower recoveries using this method. Since not all matrices could be evaluated, analysts are advised to test the chosen method against appropriate reference methods. Many materials with high moisture contents do not require drying before analysis. However, antioxidants should be added if drying is required (except when freeze drying) to protect from oxidation. An appropriate grinding technique for homogenization of the test sample should be used, when necessary.

Analysis of steryl ester concentrates and steryl ester-fortified food products are outside the scope of this method since greatly increased saponification times are required.

APPARATUS

1. 50-mL round flat-bottom flasks with 24/40 joints or equivalent
2. Teflon™ flask-neck sleeves (size 24/40)
3. Hollow, full-length stopper (size 24/40), barrel-shaped head
4. Explosion-proof hot plates or equivalent (see Notes, 1)
5. Water-cooled condensers or equivalent (see Notes, 1)
6. 2-mL amber write-on autosampler vials with Teflon™-lined caps
7. Glass powder funnel
8. Analytical balance capable of reading to ± 0.0001 g or ± 0.1 mg
9. Weighing funnels
10. 1-L transparent polymethylpentene screw top flask
11. 500-mL Nalgene® bottle
12. Porous boiling chips

REAGENTS

1. Hydrochloric Acid, 1.25 M in methanol (MeOH). Do not use aqueous HCl.
2. Sodium Hydroxide in MeOH, 2.3 M analytical reagent grade. Add 92 g of NaOH to a 1-L polymethylpentene flask. Add MeOH and mix well to dissolve before diluting to volume.
3. Methanol (MeOH), high purity gas chromatographic grade
4. Sodium Chloride, analytical reagent grade, saturated. Dissolve 36 g NaCl in 100 mL distilled water.
5. Hexanes or *n*-heptane, high purity gas chromatographic grade
6. Chloroform, high purity gas chromatographic grade
7. 14% Boron Trifluoride in MeOH (see Notes, 2)
8. Sodium Sulfate, anhydrous, reagent grade
9. Pyrogallol, ACS reagent
10. Internal standard: 13:0, Tritridecanoin (P/N T-135, Nu-Chek-Prep, Inc. or equivalent) for unknowns, dairy or ruminant fats. Accurately weigh out 2.5 g of 13:0 triacylglycerol (TAG) into a weighing funnel. Empty the weighing funnel into a 500-mL class A volumetric flask and rinse the funnel with hexanes or *n*-heptane to ensure complete quantitative transfer into the flask. Add hexanes or *n*-heptane to the flask, mix to dissolve, and dilute to volume (see Calculations, 1). The 13:0 TAG internal standard solution is stable indefinitely if precautions are taken to eliminate the loss of *n*-heptane and therefore a change in the concentration of the internal standard.

Ce 2c-11 • Direct Methylation of Lipids in Foods by Acid-Alkali Hydrolysis

Table 1
Guide to test portion size and internal standard volume.

Expected Fat or Oil Concentration	Test Portion Amount (mg)	Fat or Oil Concentration (mg/mL)	Fat or Oil Amount (mg)	Amount of TAG IS Added (mg)	13:0 TAG IS (mL)	21:0 TAG IS (mL)	23:0 TAG IS (mL)
90–100%	100	18–20	90–100	4.5–10	1–2	1–2	2–5
80–89%	100	16–18	80–90	4–9	1–2	1–2	2–5
70–79%	125	17.5–20	87.5–100	4.4–10	1–2	1–2	2–5
60–69%	125	15–17.5	75–87.5	3.8–8.8	1–2	1–2	2–5
50–59%	150	15–18	75–90	3.8–9	1–2	1–2	2–5
40–49%	200	16–20	80–100	4–10	1–2	1–2	2–5
30–39%	250	15–20	75–100	3.8–10	1–2	1–2	2–5
20–29%	325	13–19.5	65–97.5	3.3–9.8	1–2	1–2	2–5
10–19%	500	10–20	50–100	2.5–10	0.5–2	0.5–2	1–5
ND–9%	1000	<20	<100	<10	0.5–2	0.5–2	1–5

Table 2
Results of *cis*-, *trans*-, saturated, monounsaturated, and polyunsaturated fatty acid determination by 9 laboratories in a collaborative study organized by AOCS in 2011. FAMES were analyzed using AOCS Ce1j-07.

Anhydrous Milk Fat	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	8	9	7	7	8	7	9	8
Mean	91.90	87.36	56.26	5.08	26.39	22.03	3.78	3.14	0.52	2.60
Repeatability (Sr)	1.67	1.58	1.38	0.65	0.85	0.69	0.17	0.08	0.07	0.11
%RSDr	1.82	1.81	2.45	12.84	3.21	3.15	4.59	2.55	13.72	4.33
r (2.8xSr)	4.68	4.43	3.86	1.83	2.37	1.94	0.49	0.22	0.20	0.32
Reproducibility (SR)	2.63	2.52	2.14	1.16	1.57	1.36	0.33	0.21	0.11	0.22
%RSDR	2.86	2.88	3.81	22.92	5.96	6.15	8.75	6.78	21.35	8.44
R (2.8xSR)	7.37	7.05	6.00	3.26	4.41	3.80	0.93	0.60	0.31	0.61
Tallow	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	7	7	7	7	9	9	9	9
Mean	97.65	93.28	44.53	6.86	45.57	39.30	2.64	1.97	0.34	1.64
Repeatability (Sr)	1.50	1.44	0.78	0.15	0.63	0.51	0.08	0.06	0.01	0.06
%RSDr	1.54	1.54	1.75	2.21	1.39	1.31	3.09	3.18	3.35	3.61
r (2.8xSr)	4.21	4.02	2.18	0.42	1.77	1.44	0.23	0.18	0.03	0.17
Reproducibility (SR)	2.93	2.81	1.35	0.56	1.29	1.11	0.33	0.25	0.10	0.19
%RSDR	3.00	3.01	3.03	8.12	2.84	2.83	12.61	12.45	29.90	11.78
R (2.8xSR)	8.21	7.86	3.78	1.56	3.62	3.11	0.93	0.69	0.28	0.54
Chocolate cake mix	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	7	7	8	7	8	8	8	8
Mean	10.73	10.26	4.44	0.88	3.13	2.44	2.63	2.46	0.22	2.26
Repeatability (Sr)	0.23	0.22	0.10	0.03	0.09	0.07	0.05	0.04	0.03	0.04
%RSDr	2.16	2.17	2.14	3.20	2.73	2.75	1.74	1.52	14.72	1.60
r (2.8xSr)	0.65	0.62	0.27	0.08	0.24	0.19	0.13	0.10	0.09	0.10
Reproducibility (SR)	0.69	0.66	0.32	0.10	0.20	0.13	0.11	0.09	0.03	0.10
%RSDR	6.39	6.38	7.16	11.93	6.35	5.42	4.33	3.53	15.90	4.26
R (2.8xSR)	1.92	1.83	0.89	0.29	0.56	0.37	0.32	0.24	0.10	0.27

Ce 2c-11 • Direct Methylation of Lipids in Foods by Acid-Alkali Hydrolysis

Table 2
(Continued)

Cheese Powder	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	8	7	7	8	9	9	7	8
Mean	28.70	27.35	13.13	6.99	13.68	6.80	1.18	0.93	0.14	0.79
Repeatability (Sr)	0.80	0.77	0.24	0.16	0.31	0.15	0.08	0.04	0.00	0.02
%RSDr	2.79	2.80	1.83	2.29	2.29	2.24	7.19	4.63	1.94	2.46
r (2.8xSr)	2.24	2.15	0.67	0.45	0.88	0.43	0.24	0.12	0.01	0.05
Reproducibility (SR)	2.25	2.15	0.48	0.52	0.82	0.56	0.17	0.08	0.02	0.07
%RSDR	7.83	7.86	3.65	7.39	5.98	8.19	14.63	8.10	12.96	8.26
R (2.8xSR)	6.29	6.02	1.34	1.45	2.29	1.56	0.48	0.21	0.05	0.18
Extruded dog food (high protein and high fat)	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	8	7	8	8	8	8	8	8
Mean	22.23	21.24	6.67	0.31	9.75	9.40	4.80	4.75	0.38	4.37
Repeatability (Sr)	0.71	0.68	0.25	0.04	0.35	0.33	0.11	0.09	0.01	0.09
%RSDr	3.21	3.22	3.79	12.10	3.56	3.47	2.21	1.96	2.03	2.09
r (2.8xSr)	2.00	1.92	0.71	0.11	0.97	0.91	0.30	0.26	0.02	0.26
Reproducibility (SR)	1.21	1.16	0.49	0.06	0.53	0.59	0.25	0.24	0.06	0.25
%RSDR	5.45	5.45	7.28	18.79	5.45	6.26	5.28	5.03	16.23	5.71
R (2.8xSR)	3.39	3.24	1.36	0.16	1.49	1.65	0.71	0.67	0.17	0.70
Oatmeal cookie	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	7	7	7	7	7	7	7	7
Mean	19.25	18.39	9.31	0.06	7.47	7.45	2.29	2.25	0.06	2.20
Repeatability (Sr)	0.39	0.37	0.20	0.01	0.18	0.17	0.05	0.04	0.01	0.05
%RSDr	2.04	2.04	2.15	20.27	2.35	2.31	1.98	1.87	10.67	2.34
r (2.8xSr)	1.10	1.05	0.56	0.04	0.49	0.48	0.13	0.12	0.02	0.14
Reproducibility (SR)	0.65	0.62	1.05	0.03	0.87	0.86	0.10	0.09	0.01	0.08
%RSDR	3.38	3.38	11.27	47.13	11.62	11.56	4.16	3.83	18.61	3.81
R (2.8xSR)	1.82	1.74	2.94	0.08	2.43	2.41	0.27	0.24	0.03	0.23
Evaporated milk	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	9	8	8	8	9	9	9	9
Mean	5.96	5.67	3.69	0.32	1.69	1.41	0.23	0.19	0.03	0.16
Repeatability (Sr)	0.29	0.28	0.18	0.02	0.08	0.06	0.02	0.01	0.00	0.01
%RSDr	4.82	4.86	4.85	7.40	4.93	4.60	6.57	5.93	11.62	5.55
r (2.8xSr)	0.80	0.77	0.50	0.07	0.23	0.18	0.04	0.03	0.01	0.02
Reproducibility (SR)	0.70	0.67	0.44	0.05	0.19	0.15	0.03	0.02	0.01	0.02
%RSDR	11.79	11.86	12.00	17.04	11.45	10.67	14.77	12.28	31.54	12.15
R (2.8xSR)	1.97	1.88	1.24	0.15	0.54	0.42	0.10	0.06	0.02	0.05
Peanut butter	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	6	6	6	6	8	8	7	7	7	7
Mean	54.66	52.32	10.47	0.08	29.88	29.70	11.02	10.95	0.28	10.59
Repeatability (Sr)	0.84	0.80	0.22	0.01	5.53	5.83	0.25	0.13	0.02	0.12
%RSDr	1.54	1.54	2.09	9.37	18.51	19.64	2.27	1.16	5.30	1.09
r (2.8xSr)	2.35	2.25	0.61	0.02	15.49	16.33	0.70	0.36	0.04	0.32
Reproducibility (SR)	5.68	5.44	1.25	0.06	7.12	7.47	1.71	1.71	0.35	1.50
%RSDR	10.39	10.39	11.90	71.88	23.82	25.16	15.56	15.64	122.95	14.14
R (2.8xSR)	15.90	15.22	3.49	0.16	19.93	20.92	4.80	4.80	0.98	4.19

Table 2
(Continued)

Yogurt (plain)	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	7	7	8	8	8	8	7	7
Mean	5.54	5.26	3.42	0.30	1.54	1.29	0.22	0.18	0.04	0.14
Repeatability (Sr)	0.50	0.47	0.17	0.02	0.14	0.10	0.02	0.02	0.00	0.01
%RSDr	8.97	8.97	4.86	7.34	9.19	8.05	7.58	9.90	10.64	4.30
r (2.8xSr)	1.39	1.32	0.47	0.06	0.40	0.29	0.05	0.05	0.01	0.02
Reproducibility (SR)	0.73	0.69	0.46	0.04	0.18	0.14	0.03	0.03	0.01	0.02
%RSDR	13.19	13.20	13.39	13.86	11.53	10.62	16.05	15.12	25.57	12.25
R (2.8xSR)	2.04	1.94	1.28	0.12	0.50	0.38	0.10	0.08	0.03	0.05
Canned cat food	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	8	7	7	7	7	7	8	8
Mean	7.12	6.81	2.03	0.08	3.13	3.07	1.54	1.52	0.60	0.95
Repeatability (Sr)	0.53	0.51	0.21	0.01	0.27	0.26	0.08	0.08	0.11	0.07
%RSDr	7.46	7.44	10.30	7.97	8.48	8.55	5.08	5.03	17.81	7.47
r (2.8xSr)	1.49	1.42	0.59	0.02	0.74	0.73	0.22	0.21	0.30	0.20
Reproducibility (SR)	0.57	0.54	0.22	0.02	0.37	0.37	0.20	0.20	0.19	0.10
%RSDR	7.97	7.97	10.63	23.16	11.88	12.16	12.80	13.27	31.35	10.24
R (2.8xSR)	1.59	1.52	0.60	0.05	1.04	1.04	0.55	0.57	0.53	0.27
DHA/EPA fortified orange juice	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	7	9	7	7	7	7	7	7
Mean	0.10	0.10	0.03	0.00	0.03	0.03	0.04	0.04	0.02	0.02
Repeatability (Sr)	0.05	0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
%RSDr	45.70	45.70	49.36	0.00	10.61	9.02	11.07	11.07	19.67	3.46
r (2.8xSr)	0.13	0.13	0.04	0.00	0.01	0.01	0.01	0.01	0.01	0.00
Reproducibility (SR)	0.05	0.05	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.00
%RSDR	45.70	45.70	49.36	0.00	20.58	27.87	30.09	30.09	53.67	12.30
R (2.8xSR)	0.13	0.13	0.04	0.00	0.02	0.02	0.03	0.03	0.03	0.01
Butter	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	9	8	9	9	7	7	6	8
Mean	68.38	65.16	34.68	2.29	21.17	19.29	8.75	8.32	1.62	6.75
Repeatability (Sr)	3.26	3.11	2.51	0.17	2.26	1.98	0.32	0.31	0.06	0.24
%RSDr	4.76	4.77	7.23	7.32	10.69	10.24	3.65	3.75	3.89	3.57
r (2.8xSr)	9.11	8.70	7.02	0.47	6.34	5.53	0.89	0.87	0.18	0.67
Reproducibility (SR)	4.76	4.56	2.52	0.39	2.73	2.38	0.36	0.32	0.08	0.45
%RSDR	6.96	7.00	7.26	17.02	12.90	12.36	4.13	3.80	5.21	6.67
R (2.8xSR)	13.32	12.77	7.04	1.09	7.65	6.67	1.01	0.88	0.24	1.26
Whole egg powder	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	9	9	7	7	8	8	8	8
Mean	40.66	38.87	13.95	0.40	16.94	16.56	7.26	7.21	0.36	6.77
Repeatability (Sr)	0.97	0.93	0.69	0.02	0.49	0.48	0.13	0.13	0.03	0.13
%RSDr	2.39	2.39	4.94	5.60	2.90	2.93	1.77	1.83	7.51	1.96
r (2.8xSr)	2.72	2.61	1.93	0.06	1.38	1.36	0.36	0.37	0.08	0.37
Reproducibility (SR)	1.19	1.14	1.32	0.08	0.64	0.62	0.51	0.50	0.04	0.66
%RSDR	2.92	2.92	9.43	19.66	3.78	3.72	7.04	6.97	10.99	9.80
R (2.8xSR)	3.32	3.18	3.68	0.22	1.79	1.73	1.43	1.41	0.11	1.86

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Table 2
(Continued)

Full-fat soy flour flakes	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	9	8	7	7	9	9	8	9
Mean	22.72	21.73	3.74	0.02	5.00	4.99	13.20	13.18	1.61	11.60
Repeatability (Sr)	0.76	0.73	0.17	0.01	0.27	0.27	0.33	0.33	0.03	0.32
%RSDr	3.34	3.34	4.52	26.37	5.48	5.48	2.52	2.52	1.79	2.76
r (2.8xSr)	2.12	2.03	0.47	0.02	0.77	0.77	0.93	0.93	0.08	0.90
Reproducibility (SR)	2.19	2.10	0.40	0.02	0.27	0.27	1.23	1.24	0.14	1.10
%RSDR	9.65	9.65	10.69	88.64	5.48	5.48	9.33	9.37	8.99	9.52
R (2.8xSR)	6.14	5.87	1.12	0.06	0.77	0.77	3.45	3.46	0.40	3.09
Encapsulated DHA/EPA	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	7	8	7	7	8	8	7	7
Mean	55.46	53.08	17.79	0.80	13.92	13.38	20.51	20.09	17.72	2.55
Repeatability (Sr)	2.05	1.95	0.23	0.24	0.49	0.32	0.45	0.40	0.14	0.04
%RSDr	3.70	3.68	1.27	30.34	3.55	2.40	2.19	1.97	0.81	1.51
r (2.8xSr)	5.75	5.47	0.63	0.68	1.38	0.90	1.26	1.11	0.40	0.11
Reproducibility (SR)	2.72	2.60	0.74	0.24	0.75	0.67	1.88	2.01	1.60	0.76
%RSDR	4.90	4.90	4.18	30.34	5.37	5.03	9.15	10.02	9.05	29.89
R (2.8xSR)	7.61	7.29	2.08	0.68	2.09	1.88	5.26	5.63	4.49	2.14
Creamy ranch dressing	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	9	9	7	7	9	9	8	7
Mean	46.67	44.63	7.00	0.21	9.47	9.43	26.35	26.18	3.29	23.94
Repeatability (Sr)	0.87	0.84	0.15	0.02	0.20	0.20	0.63	0.63	0.07	0.45
%RSDr	1.87	1.87	2.17	10.74	2.10	2.11	2.38	2.39	2.10	1.88
r (2.8xSr)	2.44	2.34	0.43	0.06	0.56	0.56	1.76	1.75	0.19	1.26
Reproducibility (SR)	1.87	1.78	0.73	0.14	1.10	1.07	2.45	2.47	0.33	0.84
%RSDR	4.00	3.99	10.42	65.73	11.57	11.35	9.29	9.44	10.07	3.52
R (2.8xSR)	5.23	4.99	2.04	0.39	3.07	3.00	6.85	6.92	0.93	2.36
Potato chips	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	8	8	8	8	9	9	9	9
Mean	34.12	32.63	6.11	0.21	13.62	13.52	13.10	12.98	0.13	12.85
Repeatability (Sr)	1.11	1.06	0.19	0.03	0.45	0.45	0.46	0.45	0.01	0.45
%RSDr	3.24	3.25	3.05	14.12	3.31	3.30	3.50	3.50	6.11	3.50
r (2.8xSr)	3.10	2.96	0.52	0.08	1.26	1.25	1.28	1.27	0.02	1.26
Reproducibility (SR)	2.66	2.54	0.50	0.06	1.06	1.04	0.95	0.92	0.03	0.90
%RSDR	7.79	7.79	8.16	29.33	7.79	7.69	7.23	7.13	24.25	7.02
R (2.8xSR)	7.44	7.12	1.40	0.18	2.97	2.91	2.65	2.59	0.09	2.53
Cheese powder duplicate	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	8	8	7	9	9	9	9	9
Mean	29.29	27.92	13.28	6.67	13.37	6.78	1.19	0.94	0.13	0.81
Repeatability (Sr)	0.61	0.58	0.21	0.17	0.17	0.31	0.07	0.02	0.01	0.02
%RSDr	2.08	2.07	1.57	2.55	1.24	4.61	5.72	2.51	9.08	3.08
r (2.8xSr)	1.70	1.62	0.58	0.48	0.46	0.87	0.19	0.07	0.03	0.07
Reproducibility (SR)	2.73	2.61	1.48	0.87	1.31	0.87	0.19	0.14	0.03	0.11
%RSDR	9.33	9.35	11.12	13.12	9.84	12.82	16.15	14.65	25.82	13.98
R (2.8xSR)	7.65	7.31	4.14	2.45	3.68	2.43	0.54	0.39	0.10	0.32

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**Table 2
(Continued)**

Frozen cheese pizza	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	8	7	7	7	8	8	8	8
Mean	9.77	9.30	4.90	0.43	2.76	2.38	1.65	1.59	0.06	1.53
Repeatability (Sr)	0.58	0.55	0.29	0.03	0.18	0.15	0.10	0.10	0.00	0.10
%RSDr	5.91	5.91	5.83	7.85	6.57	6.38	6.35	6.24	6.42	6.26
r (2.8xSr)	1.62	1.54	0.80	0.09	0.51	0.43	0.29	0.28	0.01	0.27
Reproducibility (SR)	0.67	0.64	0.43	0.04	0.18	0.16	0.10	0.10	0.02	0.10
%RSDR	6.90	6.86	8.72	9.32	6.57	6.77	6.35	6.24	25.81	6.26
R (2.8xSR)	1.89	1.79	1.20	0.11	0.51	0.45	0.29	0.28	0.05	0.27
Peanut butter duplicate	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	7	7	6	7	6	6	6	6	6	6
Mean	54.08	51.76	10.20	0.06	32.33	32.29	8.66	8.64	0.10	8.46
Repeatability (Sr)	0.79	0.75	0.15	0.01	0.46	0.46	0.07	0.07	0.01	0.07
%RSDr	1.46	1.46	1.46	21.32	1.43	1.42	0.87	0.82	13.12	0.87
r (2.8xSr)	2.21	2.11	0.42	0.04	1.30	1.29	0.21	0.20	0.04	0.20
Reproducibility (SR)	3.14	3.01	0.83	0.04	1.73	1.72	0.63	0.63	0.10	0.51
%RSDR	5.81	5.81	8.12	63.63	5.36	5.33	7.32	7.28	99.62	6.04
R (2.8xSR)	8.80	8.42	2.32	0.11	4.85	4.82	1.77	1.76	0.29	1.43
Dry cereal fortified with flax	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	9	9	7	9	7	7	9	9	9	7
Mean	2.66	2.54	0.55	0.00	0.58	0.58	1.54	1.54	0.46	1.25
Repeatability (Sr)	0.08	0.08	0.01	0.00	0.02	0.01	0.04	0.04	0.01	0.04
%RSDr	3.03	3.04	2.65	0.00	2.70	2.44	2.40	2.40	2.60	2.86
r (2.8xSr)	0.23	0.22	0.04	0.00	0.04	0.04	0.10	0.10	0.03	0.10
Reproducibility (SR)	0.80	0.77	0.05	0.00	0.05	0.05	0.52	0.52	0.19	0.14
%RSDR	30.14	30.15	9.21	0.00	8.80	9.35	33.61	33.69	41.10	10.90
R (2.8xSR)	2.24	2.14	0.14	0.00	0.14	0.15	1.45	1.45	0.53	0.38
Horse feed	Total Fat (TAGS%)*	Total Fat (FA%)	Total Saturated	Total <i>trans</i>	Total MUFA	Total <i>cis</i> MUFA	Total PUFA**	Total PUFA**	Total n-3 PUFA**	Total n-6 PUFA**
n	8	8	8	9	7	7	8	8	8	8
Mean	3.14	3.00	0.72	0.00	0.75	0.74	1.51	1.51	0.20	1.31
Repeatability (Sr)	0.08	0.08	0.02	0.00	0.02	0.02	0.04	0.04	0.01	0.03
%RSDr	2.67	2.78	2.32	0.00	2.10	2.48	2.47	2.53	3.57	2.40
r (2.8xSr)	0.24	0.23	0.05	0.00	0.04	0.05	0.10	0.11	0.02	0.09
Reproducibility (SR)	0.26	0.25	0.06	0.00	0.05	0.05	0.16	0.16	0.03	0.13
%RSDR	8.27	8.31	7.78	0.00	7.32	7.10	10.67	10.69	15.69	10.06
R (2.8xSR)	0.73	0.70	0.16	0.00	0.15	0.15	0.45	0.45	0.09	0.37

*NLEA (Nutritional Labeling and Education Act) according to the Office of Nutritional Products, Labeling, and Dietary Supplements in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration

**Does not include CLA

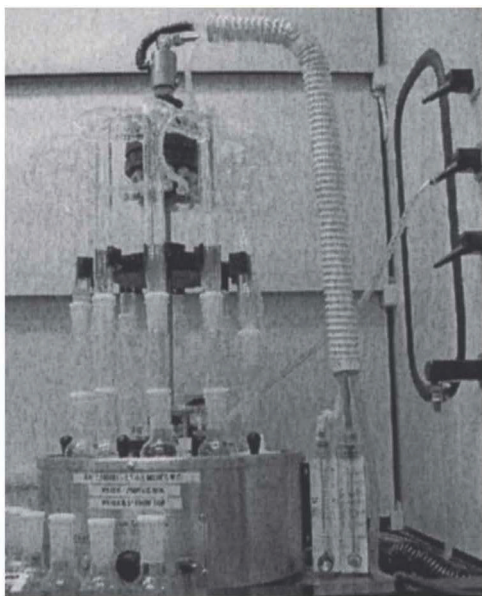


Figure 1. Example of suitable extraction apparatus (see Notes, 1)