Promulgated, Nov 28, 2013
MOHWO0014.00

Method of Test for Fatty Acids in Food

1. Scope

This method is applicable to the determination of fatty acids, trans fatty acids, saturated fatty acids, cis-monosaturated fatty acid, and cis-polysaturated fatty acids in food.

2. Method

After extraction, saponification and esterification, analytes are determined by gas chromatography (GC).

2.1. Equipment

2.1.1. Gas chromatograph.

2.1.1.1. Detector: flame ionization detector, FID.

2.1.1.2. Column: CP-Sil 88, 0.2 μm, 0.25 mm i.d. × 100 m, or an equivalent product.

2.2. Chemicals

Pyrogallic acid, reagent grade;
Ethanol (95%), reagent grade;
Hydrogen chloride, reagent grade;
Ammonia (28%), reagent grade;
Diethyl ester, reagent grade;
Petroleum ether, reagent grade;
n-Hexane, reagent grade;
Chloroform, reagent grade;
Sodium hydroxide, reagent grade;
Methanol, reagent grade;
Sodium chloride, reagent grade;
Sodium sulfate anhydrous, reagent grade;
14% Boron trifluoride in methanol, reagent grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃).

2.3. Fatty acid reference standards

2.3.1. Saturated fatty acid methyl esters: 17 items listed in **Table 1**.

2.3.2. Trans fatty acid methyl esters: 15 items listed in **Table 2**.

2.3.3. cis-Fatty acid methyl esters: 23 items listed in **Table 3**.

2.3.4. Conjugated fatty acid methyl eaters:

9-cis,11-trans-octadecadienoic methyl ester (9c,11t-18:2) and 10-trans,12-cis- octadecadienoic methyl ester (10t,12c-18:2).

2.3.5. Internal standard: triheneicosanoin (21:0).

2.4. Apparatus

2.4.1. Soxhlet apparatus.

2.4.2. Fat extractor with cap.

2.4.3. Water bath.

2.4.4. Vortex mixer.

2.4.5. Block heater: 50-200℃.

2.4.6. Glass bottle: 15 mL, amber, with Teflon cap.

2.4.7. Vail: 2 mL, amber, with cap.

2.4.8. Filter paper cylinder.

2.4.9. Filter paper.

2.4.10. Membrane filter: 0.45 μm, Nylon.

2.5. Standard solution preparation

2.5.1. Fatty acid methyl ester standard solution:

Transfer about 50 mg of fatty acid methyl ester reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with *n*-hexane to volume as the standard stock solution. When to use, dilute with *n*-hexane as the standard solution.

2.5.2. Internal standard solution:

Transfer about 100 mg of triheneicosanoin internal standard accurately weighed into a 10-mL volumetric flask, dissolve and dilute with chloroform to volume as the internal standard solution.

2.6. Reagents

2.6.1. 8.3 M hydrogen chloride:

Add 250 mL of hydrogen chloride into 110 mL of deionized water slowly, and mix wall.

2.6.2. 1 N Sodium hydroxide in methanol:

Dissolve and dilute 4 g of sodium hydroxide with methanol to 100 mL.

2.6.3. Saturated sodium chloride solution:

Take 40 g of sodium chloride, add 100 mL of deionized water, mix and then collect the supernatant.

2.7. Sample solution preparation

2.7.1. Fat extraction

2.7.1.1. General food

2.7.1.1.1. Diethyl ether extraction(note)

Transfer an accurately weighed quantity of the fine cut and homogenized sample equivalent to 100-200 mg of fat into a filter paper cylinder. Add 100 mg of pyrogallic acid and 1 mL of the internal standard solution, plug a degreasing cotton-wool above the sample, and place into the Soxhlet extractor. Add half the volume of diethyl ether into the distillation flask, and connect the distillation flask, the Soxhlet extractor, and a condenser. Heat at 60-70℃ for at least 8 hr in a water bath. Collect the extraction solution, and evaporate to dryness under vacuum at 40 ℃. Dissolve and dilute the residue with *n*-hexane to 10 mL.

Note: Diethyl ether extraction is applicable to crops, seeds, beans and easily powdered food.

2.7.1.1.2. Acid hydrolysis

Transfer an accurately weighed quantity of the fine cut and homogenized sample equivalent to 100-200 mg of fat into a flask. Add 100 mg of pyrogallic acid, 1 mL of the internal standard solution, 2 mL of ethanol and 2 granules of permutite, and mix well. Add 10 mL of 8.3 M hydrogen chloride, and mix well. Heat at 70-80℃ for 40 min in a water bath, and vortex-mix per 10 min. Then cool it, transfer into a fat extractor, and add 30 mL of ethanol and 25 mL of diethyl ether. Cap the extractor, shake for 5 min, and add 25 mL of petroleum ether. Cap the extractor, shake for 5 min, and stay for 1 hr. Transfer the upper layer into a round-bottomed flask, add 25 mL of diethyl ether and 25 mL of petroleum ether into the lower layer, and repeat the extraction procedure twice. Combined the upper layer, filter and evaporate to dryness under vacuum at 40℃. Dissolve and dilute the residue with *n*-hexane to 10 mL.

2.7.1.2. Dairy product

Transfer an accurately weighed quantity of the fine cut and homogenized sample equivalent to 100-200 mg of fat into a flask. Add 100 mg of pyrogallic acid, 1 mL of the internal standard solution, 2 mL of ethanol and 2 granules of permutite, and mix well. Add 4 mL of deionized water and 2 mL of ammonia, and mix well. Heat at 70-80℃ for 10 min in a water bath, and vortex-mix per 5 min. Then cool

it, transfer into a fat extractor, add ethanol to 30 mL, and follow the procedure described in section 2.7.1.1.2. to extract fat.

2.7.1.3. Cheese

Transfer an accurately weighed quantity of the fine cut and homogenized sample equivalent to 100-200 mg of fat into a flask. Add 100 mg of pyrogallic acid, 1 mL of the internal standard solution, 2 mL of ethanol and 2 granules of permutite, and mix well. Add 4 mL of deionized water and 2 mL of ammonia, and mix well. Heat at 70-80℃ for 20 min in a water bath, and vortex-mix per 10 min. Then Cool it, transfer into a fat extractor, add ethanol to 30 mL, and follow the procedure described in section 2.7.1.1.2. to extract fat.

2.7.1.4. Fats

Transfer 10-20 mg of the homogenized sample accurately weighed into a 1-mL volumetric flask, and add 0.1 mL of the internal standard solution. Evaporate the residual chloroform at 40℃ in a water bath, and dissolve the residue with 1 mL of *n*-hexane.

2.7.2. Saponification and esterification

Transfer 1 mL of the solution from section 2.7.1 into a glass bottle. Add 1 mL of 1 N sodium hydroxide in methanol, purge with nitrogen, and cap tightly. Vortex-mix for 30 sec, and heat at 80℃ for 15 min by a block heater for saponification. Then take the bottle out, and cool it. Add 1 mL of 14% boron trifluoride in methanol(note), purge with nitrogen, and cap tightly. Vortex-mix for 30 sec, and heat at 110℃ for 15 min by a block heater for esterification. Then take the bottle out, and cool it. Add 1 mL of *n*-hexane, cap tightly, and vortex-mix for 1 min. Add 6 mL of saturated sodium chloride solution, cap tightly, gently shake, and allow the layers to separate. Take the upper layer into a vial, add a bit of sodium sulfate anhydrous, and filter with a membrane filter. Take the filtrate as the sample solution.

Note: Because 14% boron trifluoride in methanol is a toxic chemical, performing related experiments should be in a fume hood.

2.8. Identification and quantification

Accurately inject 1 μL of the sample solution and the standard solutions into GC separately, and operate according to the following conditions. Identify each analyte based on the retention time. Calculate the amount

of fatty acids in the sample by the following formula:

2.8.1. The amount of each fatty acid methyl eater in the sample (W_{FAMEx}):

$$W_{FAMEx}\ (g) = \frac{A_x \times R_x \times W_{is} \times 1.004}{A_{is}}$$

Where,

Ax: the peak area of each fatty acid methyl ester

A_{is}: the peak area of the internal standard methyl ester

Rx: the relative response factor of each fatty acid methyl ester to the internal standard(tricosanoate) methyl ester by the FID detector, as shown in **Table 4**

W_{is}: the added amount of the internal standard (mg)

1.004: the conversion factor of the internal standard converted to its methyl ester form

2.8.2. The amount of each fatty acid in the sample (Wx):

$$Wx\ (g) = W_{FAMEx} \times F_{FAx}$$

Where,

W_{FAMEx}: the amount of each fatty acid methyl ester (g)

F_{FAx}: the conversion factor of each fatty acid methyl ester converted to its fatty acid form, as shown in **Table 5**

2.8.3. The amount of trans fatty acids in the sample:

The amount of trans fatty acids in the sample (%)

$$= \frac{\sum W_{TFAx} \times 100}{W}$$

Where,

ΣW_{TFAx}: the total amount of each trans fatty acid from section 2.8.2 (g)

W: the weight of sample (g)

2.8.4. The amount of saturated fatty acids in the sample:

The amount of saturated fatty acids in the sample (%)

$$= \frac{\sum W_{SAFAx} \times 100}{W}$$

Where,

ΣW_{SAFAx}: the total amount of each saturated fatty acid from section 2.8.2 (g)

W: the weight of sample (g)

2.8.5. The amount of cis-monounsaturated fatty acids in the sample:

The amount of cis-monounsaturated fatty acids in the sample (%)

$$= \frac{\sum W_{MUFAx} \times 100}{W}$$

Where,

ΣW_{MUFAx}: the total amount of each cis-monounsaturated fatty acid from section 2.8.2 (g)

W: the weight of sample (g)

2.8.6. The amount of cis-polyunsaturated fatty acids in the sample:

The amount of cis-polyunsaturated fatty acids in the sample (%)

$$= \frac{\sum W_{PUFAx} \times 100}{W}$$

Where,

ΣW_{PUFAx}: the total amount of each cis-polyunsaturated fatty acid from section 2.8.2 (g)

W: the weight of sample (g)

GC operating conditions(Note) :

Oven temperature program:

initial temperature: 170℃, hold for 40 min;

temperature gradient rate: 3℃/min;

final temperature: 200℃, hold for 50 min.

Detector temperature: 300℃.

Injector temperature: 250℃.

Carrier gas and flow rate: nitrogen, 0.75 mL/min.

Split ratio: 40:1.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

Further validation should be performed when interference compounds appear in samples.

Table 1. Standards of saturated fatty acid methyl esters

No.	Analyte	Abbreviation
1	tetranoic methyl ester	4:0
2	hexanoic methyl ester	6:0
3	octanoic methyl ester	8:0
4	decanoic methyl ester	10:0
5	undecanoic methyl ester	11:0
6	dodecanoic methyl ester	12:0
7	tridecanoic methyl ester	13:0
8	tetradecanoic methyl ester	14:0
9	pentadecanoic methyl ester	15:0
10	hexadecanoic methyl ester	16:0
11	heptadecanoic methyl ester	17:0
12	octadecanoic methyl ester	18:0
13	eicosanoic methyl ester	20:0
14	heneicosanoic methyl ester	21:0
15	docosanoic methyl ester	22:0
16	tricosanoic methyl ester	23:0
17	tetracosanoic methyl ester	24:0

Table 2. Standards of trans fatty acid methyl esters

No.	Analyte	Abbreviation
1	9-trans-tetradecenoic methyl ester	9t-14:1
2	9-trans-hexadecenoic methyl ester	9t-16:1
3	6-trans-octadecenoic methyl ester	6t-18:1
4	9-trans-octadecenoic methyl ester	9t-18:1
5	11-trans-octadecenoic methyl ester	11t-18:1
6	9,12-trans-octadecadienoic methyl ester	9t,12t-18:2
7	9-cis,12-trans-octadecadienoic methyl ester	9c,12t-18:2
8	9-trans,12-cis-octadecadienoic methyl ester	9t,12c-18:2
9	9,12,15-trans-octadecatrienoic methyl ester	9t,12t,15t-18:3
10	9-trans,12-trans,15-cis-octadecatrienoic methyl ester	9t,12t,15c-18:3
11	9-trans,12-cis,15-trans-octadecatrienoic methyl ester	9t,12c,15t-18:3
12	9-cis,12-trans,15-trans-octadecatrienoic methyl ester	9c,12t,15t-18:3
13	9-cis,12-cis,15-trans-octadecatrienoic methyl ester	9c,12c,15t-18:3
14	9-cis,12-trans,15-cis-octadecatrienoic methyl ester	9c,12t,15c-18:3
15	9-trans,12-cis,15-cis-octadecatrienoic methyl ester	9t,12c,15c-18:3

Table 3. Standards of cis-fatty acid methyl esters

No.	Analyte	Abbreviation
1	9-cis-tetradecenoic methyl ester	9c-14:1
2	10-cis-pentadecanoic methyl ester	10c-15:1
3	9-cis-hexadecenoic methyl ester	9c-16:1
4	10-cis-heptadecanoic methyl ester	10c-17:1
5	6-cis-octadecenoic methyl ester	6c-18:1
6	9-cis-octadecenoic methyl ester	9c-18:1
7	11-cis-octadecenoic methyl ester	11c-18:1
8	9,12-cis-octadecadienoic methyl ester	9c,12c-18:2
9	9,12,15-cis-octadecatrienoic methyl ester	9c,12c,15c-18:3
10	6,9,12-cis-octadecatrienoic methyl ester	6c,9c,12c-18:3
11	11-cis-eicosenoic methyl ester	11c-20:1
12	11,14-cis-eicosadienoic methyl ester	11c,14c-20:2
13	8,11,14-cis-eicosatrienoic methyl ester	8c,11c,14c-20:3
14	11,14,17-cis-eicosatrienoic methyl ester	11c,14c,17c-20:3
15	5,8,11,14-cis-eicosatetraenoic methyl ester	5c,8c,11c,14c-20:4
16	5,8,11,14,17-cis-eicosapentaenoic methyl ester	5c,8c,11c,14c,17c-20:5
17	13-cis-docosanoic methyl ester	13c-22:1
18	13,16-cis-docosadienoic methyl ester	13c,16c-22:2
19	7,10,13,16-cis-docosatetraenoic methyl ester	7c,10c,13c,16c-22:4
20	4,7,10,13,16-cis-docosapentaenoic methyl ester	4c,7c,10c,13c,16c-22:5
21	7,10,13,16,19-cis-docosapentaenoic methyl ester	7c,10c,13c,16c,19c-22:5
22	4,7,10,13,16,19-cis-docosahexaenoic methyl ester	4c,7c,10c,13c,16c,19c-22:6
23	15-cis-tetracosanoic methyl ester	15c-24:1

Table 4. The relative response factor of each fatty acid methyl ester to tricosanoate methyl ester by the FID detector

Fatty acid	Rx	Fatty acid	Rx	Fatty acid	Rx	Fatty acid	Rx
4:0	1.5742	14:1	1.0587	18:2	1.0087	22:1	0.9881
5:0	1.4324	15:0	1.0540	18:3	1.0017	22:2	0.9825
6:0	1.3378	15:1	1.0457	18:4	0.9949	22:3	0.9769
7:0	1.2702	16:0	1.0422	19:0	1.0142	22:4	0.9713
8:0	1.2195	16:1	1.0345	20:0	1.0067	22:5	0.9655
9:0	1.1802	16:2	1.0267	20:1	1.0005	22:6	0.9599
10:0	1.1486	16:3	1.0189	20:2	0.9943	23:0	0.9882
11:0	1.1228	16:4	1.0111	20:3	0.9880	24:0	0.9830
12:0	1.1013	17:0	1.0318	20:4	0.9819	24:1	0.9779
12:1	1.0910	17:1	1.0244	20:5	0.9665		
13:0	1.0831	18:0	1.0225	21:0	1.0000		
14:0	1.0675	18:1	1.0155	22:0	0.9939		

Table 5. The conversion factor of each fatty acid methyl ester converted to its fatty acid form

Fatty acid	F_{FAx}	Fatty acid	F_{FAx}	Fatty acid	F_{FAx}
4:0	0.8627	17:0	0.9507	21:0	0.9588
6:0	0.8923	17:1	0.9503	22:0	0.9604
8:0	0.9114	18:0	0.9530	22:1	0.9602
10:0	0.9247	18:1	0.9527	22:2	0.9600
11:0	0.9300	18:2	0.9524	22:3	0.9598
12:0	0.9346	18:3	0.9520	22:4	0.9595
13:0	0.9386	18:4	0.9517	22:5	0.9593
14:0	0.9421	20:0	0.9570	22:6	0.9590
14:1	0.9417	20:1	0.9568	23:0	0.9620
15:0	0.9453	20:2	0.9565	24:0	0.9633
15:1	0.9449	20:3	0.9562	24:1	0.9632
16:0	0.9481	20:4	0.9560		
16:1	0.9477	20:5	0.9557		