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PEOPLE'S REPUBLIC OF CHINA

GB 5009.168-2016

National food safety standard

Determination of fatty acids in foods

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Foreword

This Standard replaces GB/T 5009.168-2003 “Determination of eicosapentaenoic acid and docosahexaenoic acid in foods”, GB/T 22223-2008 “Determination of total fat, saturated fat, and unsaturated fat in foods - Hydrolytic Extraction - Gas Chromatography”, GB 5413.27-2010 “National food safety standard - Determination of fatty acids in foods for infants and young children milk and milk products”, GB/T 9695.2-2008 “Meat and meat products - Determination of fatty acids”, GB/T 17376-2008 “Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids”, GB/T 17377-2008 “Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids”, SN/T 2922-2011 “Determination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in foods for export - Gas chromatography”, NY/T 91-1988 “Determination of erucic acid in the oil of rapeseed - Gas chromatographic method”.

Compared with GB/T 5009.168-2003, the main changes of this Standard are as follows:

- the standard name is changed to “National food safety standard - Determination of fatty acids in foods”;

- ADD the internal standard method and the normalization method;

- MODIFY the chromatographic column in the original standard, changing the glass column to capillary chromatographic column.

National food safety standard

Determination of fatty acids in foods

1 Scope

This Standard specifies the determination method for the content of fatty acids in foods.

This Standard applies to the determination of total fats, saturated fats (fatty acids) and unsaturated fats (fatty acids) in foods.

In this Standard, the hydrolysis-extraction method applies to the determination of the content of fatty acids in foods; the transesterification method applies to the determination of the content of fatty acids in fat samples with free fatty acid content of not more than 2 %; the acetyl chloride-methanol method applies to the determination of the content of fatty acids in milk powder and anhydrous cream samples with water content less than 5 %.

Method I Internal standard method

2 Principle

2.1 Hydrolysis-extraction method: after the fats in the sample that has added with internal standard are extracted with hydrolysis-ether solution, saponify and methyl-esterify the sample under alkaline conditions to produce fatty acid methyl esters. After analyzed by capillary column gas chromatography, use internal standard method to quantitatively determine the content of fatty acid methyl esters. According to the content and conversion coefficients of various fatty acid methyl esters, calculate the content of total fats, saturated fats (fatty acids), monounsaturated fats (fatty acids) and polyunsaturated fats (fatty acids).

Animal and vegetable fat samples are directly saponified and fatty-acid-methyl esterified after adding with internal standard, without fat extraction.

2.2 Transesterification method (applies to fats with free fatty acid content of not more than 2 %): DISSOLVE fats in isooctane; after adding internal standard, ADD potassium hydroxide methanol solution to make the sample methyl-esterified through transesterification. After the reaction is complete, USE sodium sulfate to neutralize the residual potassium hydroxide to avoid saponification of methyl esters.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytical regents and the water is the Grade 1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl).

3.1.2 Ammonia ($NH_3 \cdot H_2O$).

3.1.3 Pyrogallic acid ($C_6H_6O_3$).

3.1.4 Ether ($C_4H_{10}O$).

3.1.5 Petroleum ether: with a boiling range of 30 °C ~ 60 °C.

3.1.6 Ethanol (C_2H_6O) (95 %).

3.1.7 Methanol (CH_3OH): chromatographically pure.

3.1.8 Sodium hydroxide (NaOH).

3.1.9 N-heptane [$CH_3(CH_2)_5CH_3$]: chromatographically pure.

3.1.10 Boron trifluoride methanol solution, with a concentration of 15 %.

3.1.11 Anhydrous sodium sulfate (Na_2SO_4).

3.1.12 Sodium chloride (NaCl).

3.1.13 Isooctane [$(CH_3)_2CHCH_2C(CH_3)_3$]: chromatographically pure.

3.1.14 Sodium bisulfate ($NaHSO_4$).

3.1.15 Potassium hydroxide (KOH).

3.2 Preparation of reagents

3.2.1 Hydrochloric acid solution (8.3 mol/L): WEIGH 250 mL of hydrochloric acid; DILUTE with 110 mL of water; MIX well. It can be placed for 2 months at room temperature.

3.2.2 Ether-petroleum ether mixture (1 + 1): TAKE the same volume of ether and petroleum ether; MIX well for further use.

3.2.3 Sodium hydroxide methanol solution (2 %): TAKE 2 g of sodium hydroxide and

to the walls of the flask are mixed into the solution. After the hydrolysis is complete, REMOVE the flask and COOL to room temperature.

Acid and alkali hydrolysis method: cheeses: ADD 5 mL of ammonia, MIX well. PLACE the flask in a water bath at 70 °C to 80 °C to hydrolyze for 20 min. SHAKE the flask once every 5 min so that the particles adhering to the walls of the flask are mixed into the solution. Then ADD 10 mL of hydrochloric acid, CONTINUE to hydrolyze for 20 min. SHAKE the flask once every 10 min so that the particles adhering to the walls of the flask are mixed into the solution. After the hydrolysis is complete, REMOVE the flask and COOL to room temperature.

5.2.1.3 Fat extraction

ADD 10 mL of 95 % ethanol to the hydrolyzed sample, MIX well. TRANSFER the hydrolyzate in the flask to a separatory funnel; USE 50 mL of ether petroleum ether mixture to rinse the flask and stopper; ADD the rinsing solution to the separatory funnel; COVER the cap. SHAKE for 5 min, STAND for 10 min. COLLECT the ether layer extract to a 250-mL flask. EXTRACT the hydrolyzate three times according to the above procedure. Finally, USE ether petroleum ether mixture to rinse the separatory funnel and COLLECT the rinsing solution in a 250-mL flask. USE rotary evaporator to concentrate to dryness and the residue is the fat extract.

5.2.1.4 Saponification of fats and methyl esterification of fatty acids

ADD 8 mL of 2 % sodium hydroxide methanol solution in the fat extract; CONNECT to the reflux condenser; REFLUX in 80 °C ± 1 °C water bath until the oil droplets disappear. ADD 7 mL of 15 % boron trifluoride methanol solution from the top of the reflux condenser and continue to REFLUX at 80 °C ± 1 °C for 2 min. RINSE the reflux condenser with a small amount of water. STOP heating, REMOVE the flask from the water bath and quickly COOL to room temperature.

Accurately ADD 10 mL ~ 30 mL of n-heptane; SHAKE for 2 min; ADD saturated sodium chloride solution; STAND for stratification. PIPETTE about 5 mL of the upper n-heptane extract to a 25-mL test tube; ADD about 3 g ~ 5 g of anhydrous sodium sulfate; SHAKE for 1 min; STAND for 5 min; PIPETTE the supernatant to the injection bottle for determination.

5.2.2 Transesterification method

Applies to fat samples with free fatty acid content of not more than 2 %.

5.2.2.1 Weighing of samples

WEIGH 60.0 mg of sample to a test tube with a stopper, accurate to 0.1 mg, and accurately ADD 2.0 mL of internal standard solution.

milligrams per milliliter (mg/mL);

A_{11} - the peak area of methyl undecanoate;

A_{Si} - the peak area of fatty acid methyl ester *i*;

ρ_{11} - the concentration of methyl undecanoate in the mixed standard, in milligrams per milliliter (mg/mL).

6.2 Content of saturated fats (fatty acids) in the sample

The content of saturated fats (fatty acids) in the sample is calculated according to equation (3), and the content of single saturated fatty acids in the sample is calculated according to equation (4):

$$X_{\text{Saturated Fat}} = \sum X_{\text{SFA}i} \quad \cdots\cdots(3)$$

$$X_{\text{SFA}i} = X_{\text{FAME}i} \times F_{\text{FAME}i\text{-FA}i} \quad \cdots\cdots(4)$$

where:

$X_{\text{Saturated Fat}}$ - the content of saturated fats (fatty acids), in grams per gram (g/100 g);

X_{SFAi} - the content of single saturated fatty acids, in grams per gram (g/100g);

X_{FAMEi} - the content of single saturated fatty acid methyl esters, in grams per gram (g/100 g);

$F_{\text{FAMEi-FAi}}$ - the coefficient of conversion from fatty acid methyl esters to fatty acids.

The coefficient of conversion from fatty acid methyl esters to fatty acids $F_{\text{FAMEi-FAi}}$ is shown in Annex D. The coefficient of conversion from fatty acid methyl ester *i* to fatty acids is calculated according to equation (5):

$$F_{\text{FAME}i\text{-FA}i} = \frac{M_{\text{FA}i}}{M_{\text{FAME}i}} \quad \cdots\cdots(5)$$

where:

$F_{\text{FAMEi-FAi}}$ - the coefficient of conversion from fatty acid methyl esters to fatty acids;

M_{FAi} - the molecular weight of fatty acid *i*;

M_{FAMEi} - the molecular weight of fatty acid methyl ester *i*.

6.3 Content of monounsaturated fats (fatty acids) in the sample

The content of monounsaturated fats (fatty acids) in the sample is calculated according

esterified without fat extraction.

7.2 Acetyl chloride-methanol method (applies to milk powder and anhydrous cream samples with water content of less than 5 %): acetyl chloride reacts with methanol to produce hydrochloric acid-methanol and make the fats and free fatty acids methyl esterified. After extracted with toluene, separately tested with gas chromatography, and quantify with external standard method.

7.3 Transesterification method (applies to fats with free fatty acid content of not more than 2 %): DISSOLVE the fat sample in isooctane; ADD potassium hydroxide methanol solution to make the sample methyl-esterified through transesterification. After the reaction is complete, NEUTRALIZE the residual potassium hydroxide with sodium sulfate, and USE external standard method to quantitatively determine the content of fatty acids.

8 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytical regents and the water is the Grade 1 water specified in GB/T 6682.

8.1 Reagents

8.1.1 Hydrochloric acid (HCl).

8.1.2 Ammonia ($NH_3 \cdot H_2O$).

8.1.3 Pyrogallic acid ($C_6H_6O_3$).

8.1.4 Ethyl ether ($C_4H_{10}O$).

8.1.5 Petroleum ether: with a boiling range of 30 °C ~ 60 °C.

8.1.6 Ethanol (C_2H_6O) (95 %).

8.1.7 Methanol (CH_3OH): chromatographically pure.

8.1.8 Sodium hydroxide (NaOH).

8.1.9 N-heptane [$CH_3(CH_2)_5CH_3$]: chromatographically pure.

8.1.10 Boron trifluoride methanol solution: with a concentration of 15 %.

8.1.11 Anhydrous sodium sulfate (Na_2SO_4).

8.1.12 Sodium chloride (NaCl).

8.1.13 Anhydrous sodium carbonate (Na_2CO_3).

PREPARE the standard working solution of proper concentration with toluene. STORE it in the refrigerator below -10 °C, valid for 3 months.

9 Apparatus and equipment

9.1 Homogenizer or laboratory tissue pulverizer or grinder.

9.2 Gas chromatograph: with a hydrogen flame ion detector (FID).

9.3 Capillary chromatographic column: with polydipyldiphenyl siloxane strong polar stationary phase, a column length of 100 m, an inner diameter of 0.25 mm and a film thickness of 0.2 μm.

9.4 Constant temperature water bath: with a temperature range of 40 °C ~ 100 °C, temperature control ± 1 °C.

9.5 Analytical balance: with a division of 0.1 mg.

9.6 Centrifuge: with a speed ≥ 5000r/min.

9.7 Rotary evaporator.

9.8 Screw glass tube (with a screw cap with inner lining made of PTFE): 15 mL.

9.9 Centrifuge tube: 50 mL.

10 Analysis procedure

10.1 Preparation of samples

The procedure is the same as 5.1.

10.2 Pretreatment of samples

10.2.1 Hydrolysis-extraction method

10.2.1.1 Weighing of samples

WEIGH 0.1 g ~ 10 g (accurate to 0.1 mg, containing100 mg ~ 200 mg of fat) of uniform sample into a 250-mL flat-bottomed flask; ADD about 100 mg of pyrogallic acid; ADD several zeolites; ADD 2 mL of 95 % ethanol; MIX well. According to the type of sample, select different hydrolysis methods.

10.2.1.2 Hydrolysis of samples

The procedure is the same as 5.2.1.2.

11 Expression of analysis results

11.1 Content of each fatty acids in samples

QUANTIFY by the peak area of chromatographic peaks. The content of each fatty acids in the sample is calculated according to equation (12):

$$X_i = \frac{A_i \times m_{S_i} \times F_{TG_i\text{-}FA_i}}{A_{S_i} \times m} \times 100 \qquad \cdots\cdots (12)$$

where:

X_i - the content of each fatty acids in the sample, in grams per gram (g/100g);

A_i - the peak area of each fatty acid methyl ester in the sample determination solution;

m_{Si} - the mass of the standard contained in fatty acid triglyceride standard working solution drawn in the preparation of the standard determination solution, in milligrams (mg);

$F_{TGi\text{-}FAi}$ - the coefficient of conversion from each fatty acid triglyceride to fatty acids, see Annex D;

A_{Si} - the peak area of each fatty acid in the standard determination solution;

m - the mass of the sample, in milligrams (mg);

100 - the coefficient of conversion from the content to the content in per 100 g of the sample.

11.2 Content of total fatty acids in the sample

The content of total fatty acids in the sample is calculated according to equation (13):

$$X_{\text{Total FA}} = \sum X_i \qquad \cdots\cdots (13)$$

where:

$X_{\text{Total FA}}$ - the content of total fatty acids in the sample, in grams per gram (g/100 g);

X_i - the content of each fatty acid in the sample, in grams per gram (g/100 g).

The result retains 3 significant digits.

Method III Normalization method

15 Analysis procedure

15.1 Preparation of samples

The procedure is the same as 5.1.

15.2 Hydrolysis-extraction method

15.2.1 Weighing of samples

WEIGH 0.1 g ~ 10 g (accurate to 0.1 mg, containing100 mg ~ 200 mg of fat) of uniform sample into a 250-mL flat-bottomed flask; ADD about 100 mg of pyrogallic acid; ADD several zeolites; ADD 2 mL of 95 % ethanol; MIX well. According to the type of sample, select different hydrolysis methods.

15.2.2 Hydrolysis of samples

The procedure is the same as 5.2.1.2.

15.2.3 Fat extraction

The procedure is the same as 5.2.1.3.

15.2.4 Saponification of fats and methyl esterification of fatty acids

The procedure is the same as 5.2.1.4.

15.2.5 Chromatographic determination

The chromatographic reference conditions are the same as 5.3.1.

15.3 Transesterification method

15.3.1 Weighing of samples

WEIGH 60.0 mg of sample into a test tube with a stopper, accurate to 0.1 mg.

15.3.2 Preparation of methyl ester

The same as 5.2.2.2.

16 Expression of analysis results

The percentage ratio of a fatty acid in the sample to total fatty acids Y_i is calculated according to equation (14). By determining the percentage ratio of the corresponding peak area to the sum of the peak area of all components, calculate the content of a

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