National Food Safety Standard of P. R. of China

GB 5413 .3– 2010

National Food Safety Standard

Determination of fat in foods for infant and young children, milk and milk products

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Preface

This standard replaces the determination of fat under GB/T 5009.46-2003<Method of analysis of hygienic standard of milk and milk products>, the determination of fat under GB/T 5409-85<Analytical method for milk>, the determination of fat under GB/T 5416-85<Analytical method for butter> and GB/T 5413.3- 1997 <Milk powder and formula foods for infant and young children-determination of fat>.

Appendix A is informative.

The previous editions to be replaced by this standard:

- GB/T 5409-85
- GB/T 5413.3 - 1997
- GB/T 5416- 85
- GB/T 5009.46-1985, GB/T 5009.46-1996, GB/T 5009.46-2003

Milk powder and formula foods for infant and young children Determination of fat

1. Scope

This standard is applicable for the determination of fat in pasteurized milk, UHT milk, raw milk, fermented milk, modified milk, milk powder, evaporated milk, sweetened condensed milk and formulated condensed milk, cream butter and anhydrous milkfat, cheese, processed cheese and infant formula.

Method 1 in this standard is applicable for pasteurized milk, UHT milk, raw milk, fermented milk, modified milk, milk powder, evaporated milk, sweetened condensed milk and formulated condensed milk, cream butter and anhydrous milkfat, cheese, processed cheese and infant formula.

Method 2 in this standard is applicable for pasteurized milk, UHT milk and raw milk.

2. Normative documents

The normative documents are indispensable to this standard. For dated reference, only the dated reference apply. For undated references, the latest edition of the normative documents referred to applies.

Method I

3. Priciple

Extraction sample ethanol-ammonia solution by diethyl-ether and petroleum ether, distillation or evaporation, determine the mass of extraction in ether.

4. Reagents and materials

All reagents, if not specified, are A.R; all water, if not specifly, are third class water as classified in GB/T 6682.

4.1 Amylase: enzyme activity ≥ 1.5 U/mg

4.2 Ammonia solution(NH_4OH): 25%(w/V)

Note: Higher concentration of ammonia solution allowed.

4.3 Ethanol (C_2H_5OH) : Minimum 95% (v/v)

4.4 Aether ($C_4H_{10}O$): Does not contain peroxide, antioxidant and meet the request for testing.

4.5 Petroleum ether(C_nH_{2n+2}): boiling degree 30 - 60 □

4.6 Mix-reagents: Prepare the mixture of diethyl-ether (4.4) and petroleum ether (4.5) with equal volume before using

4.7 Iodine solution (I_2): approximately 0.1 mol/L

4.8 Congo-red solution ($C_{32}H_{22}N_6Na_2O_6S_2$): dissolve 1g Congo-red into water, dilute to 100ml.

Note: selective using. Congo-red can mark the interface of solvent and water, other solution also can be used if it can coloration the water phase and do not affect the result.

4.9 Hydrochloric acid (6 mol/L): Weigh 50 mL hydrochloric acid to pour slowly into 40 mL water for a mixed solution to 100 mL.

5. Equipment and Apparatus

5.1 Analysis balance: Precision to 0.1mg

5.2 Centrifuge: To emplace the Maj. Flask or tube, rev: 500-600r/min, which can bring 80-90g gravity field.

5.3 Drying Oven

5.4 Water bath

5.5 Maj. Flask: With high quality cork stopper or other material (eg silica gel or Polytetrafluoroethylene) which doe not affect the solvent. Firstly immersed cork stopper in diethyl-ether , then put into the water with temperature at 60□ or above for minimum 15min, cool down with water, before using. Immersed the cork stopper in water when not using and change the immersing water daily.

Note: also can use a flask with siphon, see appendix A. The shape of the bottom of the pipe in the flask is like a spoon.

6. Test Procedure

6.1 Preparation of the fat collection bottle

Put a few zeolites in a dry fat collection bottle and leave it in the dry oven for 1 hour. Cool the bottle to room temperature, weigh to precision 0.1 mg/g.

Note: The fat collection bottle at own choice.

6.2 Blank test

Conduct the blank test together with the sample test following the same steps and using reagents, but using 100ml water instead of the diluted sample.

6.3 Determination

6.3.1 Pasteurized milk,UHT milk, raw milk, fermented milk,modified milk

Weigh 10g well mixed sample (precision to 0.0001 g) into Maj. flask.

6.3.1.1 Mix with 2ml ammonia solution(4.2) and immediately put the flask into water bath at 65□±5□ ; heat up for 15-20min and shaking constantly; Cool down to room temperature and rest 30s before next step.

6.3.1.2 Mix with 10ml ethanol (4.3) absolutely and slowly and avoid the solution too close to the bottle-neck. If necessary, add 2 drops of Congo red solution (4.8).

6.3.1.3 Add 25ml diethyl-ether (4.4), put on the cork the stopper and keep the flask in horizontal position. Clip on the shake machine with the small ball and shake100 times/ min or shake manually but avoid forming emulsified solution). Carefully open the cork when the flask cools down. Washing the cork and bottlenecks with small volume of mix-reagents and flush the fluid into the flask.

6.3.1.4 Add 25ml petroleum ether (4.), stuffed with re-wetting cork and follow the procedure as 6.3.1.3, gently shaking for 30 seconds.

6.3.1.5 Put the corked flask into centrifuge for 5 minutes with 500 ~ 600 rotation /minute . Otherwise rest the flask for at minimum 30min until the up layer liquid is clear and distinguishable separated from the water layer.

6.3.1.6 Carefully open the cork, flush cork and bottlenecks wall with small volume of mixed solvent and flow the solvent into the flask.

If the two-phase interface below the junction of small ball and bottle bottle, then add water slowly along the sides of the bottle to make liquid higher than the junction of small ball and body of bottle (see Figure 1) for easily pouring.

6.3.1.7 Pour as much the upper layer into the fat-collection bottle which containing zeolites to avoid pouring water layer out. (See Figure 2)

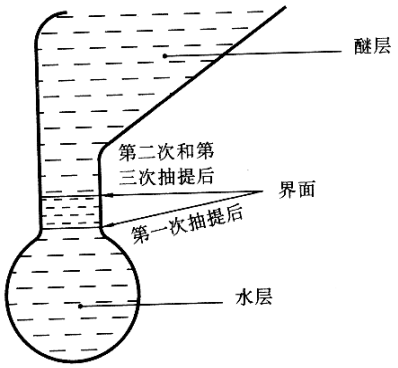


Figure 1 Before pour the layer of ether

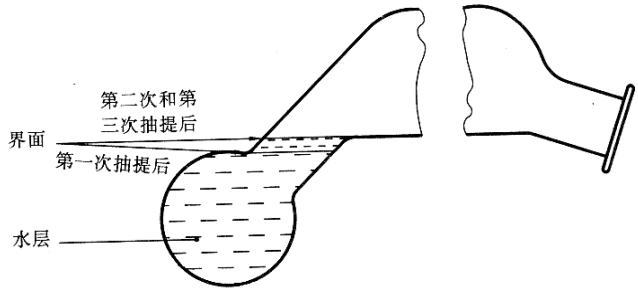


Figure 2 After pour the layer of ether

6.3.1.8 Wash the outside of bottlenecks with a small volume of mixed solvent and flush the fluid into the fat-collection bottle. Operate carefully to prevent the solvents splashing outside of flask.

6.3.1.9 Add 5mL of ethanol into the flask to wash bottleneck wall, mixed as described in 6.3.1.2. Repeat the procedure from 6.3.1.3 - 6.3.1.8 for the second extraction but with only 15ml diethyl-ether and 15 ml petroleum ether.

6.3.1.10 Repeat the procedure from 6.3.1.2 - 6.3.1.8 for the third extraction but with only 15ml diethyl-ether and 15 ml petroleum ether.

Note: If the fat mass fraction is less than 5%, a third extraction may be omitted.

6.3.1.11 Combine all the extraction and remove the solvent in the fat collection bottle either by distillation or evaporation. Wash the inner side of bottleneck with small amount with mixed solvent before distillation.

6.3.1.12Heat the fat-collection bottle in the oven at 102 ℃ ± 2 ℃(4.4) for 1 hour. Remove the collection bottle from the oven and cool down to room temperature before weighing, precision to 0.1mg.

6.3.1.13 Repeat the operation of 6.3.1.12 until the difference of two consecutive weighing is within 0.5mg. Write down the minimum mass of fat collecting bottle and Extracts.

6.3.1.14 Add 25ml petroleum ether in fat collection bottle, slightly heat and shake until all fats dissolved as a means to verify wheather all the extracts were dissolved.

If extracts are all dissolved in the petroleum ether, then the difference of final mass value of collection bottles and the initial value is the content of fat.

6.3.1.15 If extracts are not fully dissolved in petroleum ether or not sure whether all extracts are fat, then wash with warm petroleum ether. Carefully pour out petroleum ether and leave all insoluble matter inside the bottle. Repeat this procedure for minimum three times and rinse the collection bottle by petroleum ether.

The last step is to rinse the collection bottle wtih mixed solvent and prevent splashing the solution outside of bottles. Heat the collect bottles into drying oven 102 ℃ ± 2 ℃ for 1 hour as described6.3.1.12 & 6.3.1.13.

6.3.1.16 The difference between value from 6.3.1.13 and 6.3.1.15 is the content of fat.

Note: Follow the description in Appendix A when selecting flask with a siphon or wash-bottle.

6.3.2 Milk powder and milk based baby food

Weigh the well mixed sample of 1 gram(precision to 0.0001 g) for high fat milk powder, full cream milk powder, full cream sugar added milk powder and milk based baby food; Weigh 1.5 gram (precision to 0.0001 g) for skim milk powder, whey powder and cheese powder.

6.3.2.1 Sample without starch

Add 10mL water at temperature 65 ±5℃ to wash the sample into the small ball of the flask. Mix thoroughly till sample fully dispersed then leave it under running water to cool down.

6.3.2.2 Sample with starch

Mix the sample with 0.1g amylase and a magnetic stirring rod in the flask and add 8 mL - 10mL distilled water at 45 ˚C. The solution surface should not be too high in the flask. Stir with cork on and put it in the 65 ˚C water bath for 2 hours. Shake every 10 minutes. Add two droplets Iodine solution 0.1ml/L (4.7) to verify if the starch is fully hydrolyzed. It indicates fully hydrolyzation if no blue color occurs otherwise put the flask back into water bath till no blue color observed. Cool down the flask and follow the same instruction from 6.3.1.1 - 6.3.1.16.

6.3.3 Evaporated milk, sweetened condensed milk and formulated condensed milk

Take 3 -5 gram skimmed condensed milk, full cream condensed milk and partially skimmed condensed milk or 1.5 g (precision to 0.0001g) high fat condensed milk and wash into flask with 10mL distilled water till it is well mixed.

Follow the same procedure as 6.3.1.1 - 6.3.1.16.

6.3.4. Butter and cream butter

Dissolve the butter sample in warm water bath and mix thoroughly. Weigh 0.5g (precision to 0.0001g). For cream butter, weigh 1g into flask with 8mL - 10mL distilled water at 45˚C. Well mix with 2 mL ammonia.

Follow the same procedure as 6.3.1.1 - 6.3.1.14.

6.3.5 Cheese

Weigh 2g ground sample (precision to 0.0001g) into flask and add 10 mL hydrochloric acid (4.9). Well mixed and put the cork on to heat in boiling water for 20 - 30 minutes. Follow the procedure from 6.3.1.2 - 6.3.1.16.

7. Results calculation

$$X = \frac{(m1 - m2) - (m3 - m4)}{m} \cdot 100 \quad \cdots\cdots(1)$$

Where:

X --- Fat content of the sample（g/100g）

M - Mass of the sample in gram

m1 - Mass of the extracts and collection bottles as 6.3.1.13 in gram

m2 – Mass of fat-collection bottle or if with insoluble substance the mass of the insoluble extract and fat collection bottle as measured 6.3.1.15 in gram;
m3 - Mass of the fat collection bottle and the extracts measured in blank test as 6.3.1.13 in gram;
m4 - Mass of fat-collection bottle or if with insoluble substance the mass of the insoluble extract and fat collection bottle in blank test as measured 6.3.1.15 in gram;

The result should be expressed in the arithmetic mean value of two independent tests under repeated condition with 3 significant digits.

8 Precision

The difference between two independent tests under repeated condition should with below range:

Fat content	Allowed difference
≥ 15%	≤ 0.3 g/ 100g
5 - 15%	≤ 0.3 g/ 100g
≤ 5%	≤ 0.1 g/100g

9. Others

Attentions during the test

9.1 Blank test examining reagent

Blank test is required to eliminate the influence from the surroundings and temperature.

To conduct the blank test, put 1g anhydrous cream into the fat-collection bottle. If necessary, add1g anhydrous cream in each 100ml solvent, then re-distillation, re-use as quickly as possible after distillation.

9.2 Parallel the sample test with blank test

Calibrate the result with the blank test value for reagents with non-volatile substances. The temperature difference between the balance chamber and fat-collection bottle can affect the actual mass of the extracts. Under ideal conditions (low blank value of reagent, same temperature, sufficient cooling of

fat-collection bottle), the calibrate value is normally less than 0.5mg. In the normal test, this difference can be ignored.

If the blank test values often is over 0.5mg then distill 100 mL diethyl-ether and petroleum-ether respectively to test the content of residue of the reagent. The test value with an empty control bottle and the residue of each reagent should not be higher than 0.5 mg other replace the unqualified reagent or purify the reagent.

8.3 Peroxide test in diethyl-ether

Rinse a small glass graduated cylinder with diethyl-ether. Add 10ml diethyl-ether and 1ml freshly prepared potassium iodide solvent of 100g / L. Vibrate and rest for 1min. No yellow color should be observed for all layers. Peroxide can be tested with other appropriate methods.

Treat the reagent with follow steps three days before using to make sure no peroxide exists in the diethyl-ether when no antioxidants are used.

Cut the zinc foil into a long strip with minimum length half the bottle height. For every liter of diethyl-ether, prepare 80cm^2 zinc foils.

Before using, immerse the zinc foil into 2ml 98% sulfuric acid solution which containing 10g copper sulfate pentahydrate for 1min. Rinse the zinc foil thoroughly with water and wet copper zinc foil into the ether bottle. You can also use other methods, but shall not affect the test results.

Method 2

10 Principle

Milk fat is separated through centrifugation with the aid of sulfuric acid which destroys the protein film and milk structure. The separated volume of milk fat is read to decide the content of fat.

11 Reagent and materials

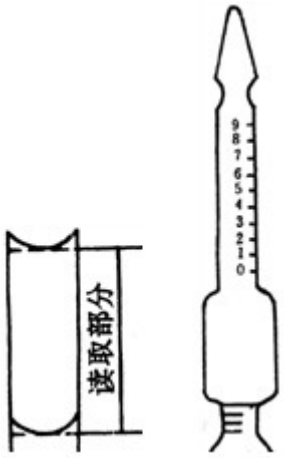
11.1 Sulfuric acid (H_2SO_4): Analytical purity, p20 around 1.84 g/L

11.2 Isoamyl alcohol ($C_5H_{12}O$): Analytical purity

12. Equipment and apparatus

12.1 Milk fat Centrifuge

12.2 Gerber butyrometer: precision 0.1%, see picture 3



Picture 3

12.3 10.75 m L Pipette

13. Procedure

Add 10mL sulfuric acid (11.1) into butyrometer. Carefully and accurately add 10.75 mL sample along the tube wall and keep it away from contact sulfuric acid. Add 1mL isoamyl alchohol (11.2) and put on the cork. Keep the lip downward and wrap the butyrometer with a piece of cloth at the same time to avoid the splash of the liquid. Shake thoroughly to the uniformly brown color and rest for a minutes (lip downward) and then leave it into water bath at 65 - 70℃ for 5 minutes. Afterwards centrifugate for 5 minutes with 1100 r/minute in the centrifugate. Put it back into water bath at 65 -70 ℃ for 5 minutes (the surface of water should be higher than the fat layer in the butyrometer. Take it out and read immediately the percentage of fat content.

14. Precision

The absolute value of two independent tests under the same condition should not

exceed 5% of the arithmetic mean value.

APPENDIX A

(Informative)

Steps of using liposuction tube with siphon or wash-bottle

A.1 Steps

A.1.1 Preparation of the fat collection bottle: refer to 6.1.

A.1.2 Blank test: refer to 6.2 & 6.9

A.1.3 Determination

A.1.3.1 For pasteurized milk,UHT milk, raw milk, fermented milk,modified milk, weigh 10g (precision to 0.0001g) to the bottom of liposuction tube.

For milk powder and milk based baby food: Weigh 1g for well mixed high fat milk powder, full cream milk fat, sugar added full cream milk powder and milk based baby food; Weigh 1.5g (precision to 0.0001g) for skim milk powder, whey powder, cheese powder to the bottom of tube. Add 10ml water (65 ℃ ± 5 ℃) into the samples. Mix thoroughly with the sample till the sample is completely dispersed the leave it under running water for cooling.

Condensed milk: Weigh 10g for skimmed condensed milk, 3 -5 g for full cream condensed milk and partly skimmed condensed milk, 1.5 g (precision to 0.001g) for high fat condensed milk to the bottom of tube. Mixed well with 10mL water.

Butter and cream butter: Dissolve the sample in warm water. Weigh 0.5g (precision to 0.001g) well mixed sample to the bottom of the tube. Follow A.1.3.1.1 for above samples.

Cheese: Weigh 2g ground sample (precision to 0.001g). Mix with 9mL water and 2mL ammonia. Stir well with glass rob then slightly heat up to dissolve the casein. Neutralize with hydrochloric acid(4.9) and then add 10 mL hydrochloric acid (4.9) and 0.5 g sea sand. Put on the lid and boil for 5 minutes. Move the substance from the beaker to the bottom of the tube. Wash the beaker with 25 mL diethyl ether and pour it into the tube. Follow the procedure A.1.3.1.4" Put on the cork..."

A.1.3.1.1 Add 2ml ammonia solution (3.2) to thoroughly mix with the diluted sample at the bottom of the tube. Imediately proceed to the next step after adding ammonia.

A.1.3.1.2 Place the liposuction tube in water bath (65 ℃ ± 5 ℃) and heat for 15 ~ 20 minutes. Shake the sample tube occasionally and then cool down to room temperature.

A.1.3.1.3 Add 10ml ethanol to mix thoroughly with the sample at the bottom of the tube. When necessary, add 2 drops of Congo-red solution.

A1.3.1.4 Add 25ml diethyl-ether, put on cork (saturated with water) or other corks soaked by water, reverse up and down for 1min. Watch out and avoid formation of the long lasting emulsions. When necessary, cool the tube with the running water. Open the cork carefully. Rinse the cork and the tube neck with a small volume of mixed solvent (using the wash-bottle) and flush fluid into the tube.

A1.3.1.5 Add 25ml petroleum ether, put on the cork (wet with water). Gently shaking for 30s as A.1.3.1.3.

A1.3.1.6 Placed corked tubes in the centrifuge. Centrifugate for 1 - 5 minutes with 500 ~ 600r/min. Alternatively rest the tube for minimum 30min until the top layer is clear and obviously separated from the water, cool down,

A1.3.1.7 Carefully open the cork. Rinse the cork and the tube neck with a

small volume of mixed solvent. Collect the flushing fluid into the tube.

A1.3.1.8 Put siphon or wash-bottle connector into the tube, press the long branch pipes until 4mm above the two-phase interface. the Interior branch pipe should be parallel with the tube axis.

Move the upper layer liquid carefully into the collection bottle containing zeolite or intometal utensils. Avoid moving any any water phase. Rinse long branch pipe outlet with a small volume of mixed solvent and collect washing fluid in the collection bottle.

A1.3.1.9 Loose tube neck joint. Flush the joints and the internal length of the lower part of the branch pipe with a small volume of mixed solvent. Plug the joint again and flush the fluid into the tube.

Wash outlet with a small amount of mixed solvent and flushing fluid into the bottle. When necessary, remove part of the solvent by distillation or evaporation following A.6.3.1.11.

A.1.3.1.10 Release the joint at the bottle neck. Slightly raise the joint and add 5ml of ethanol. Flush long branch pipes with ethanol and then mix following A.1.3.1.3.

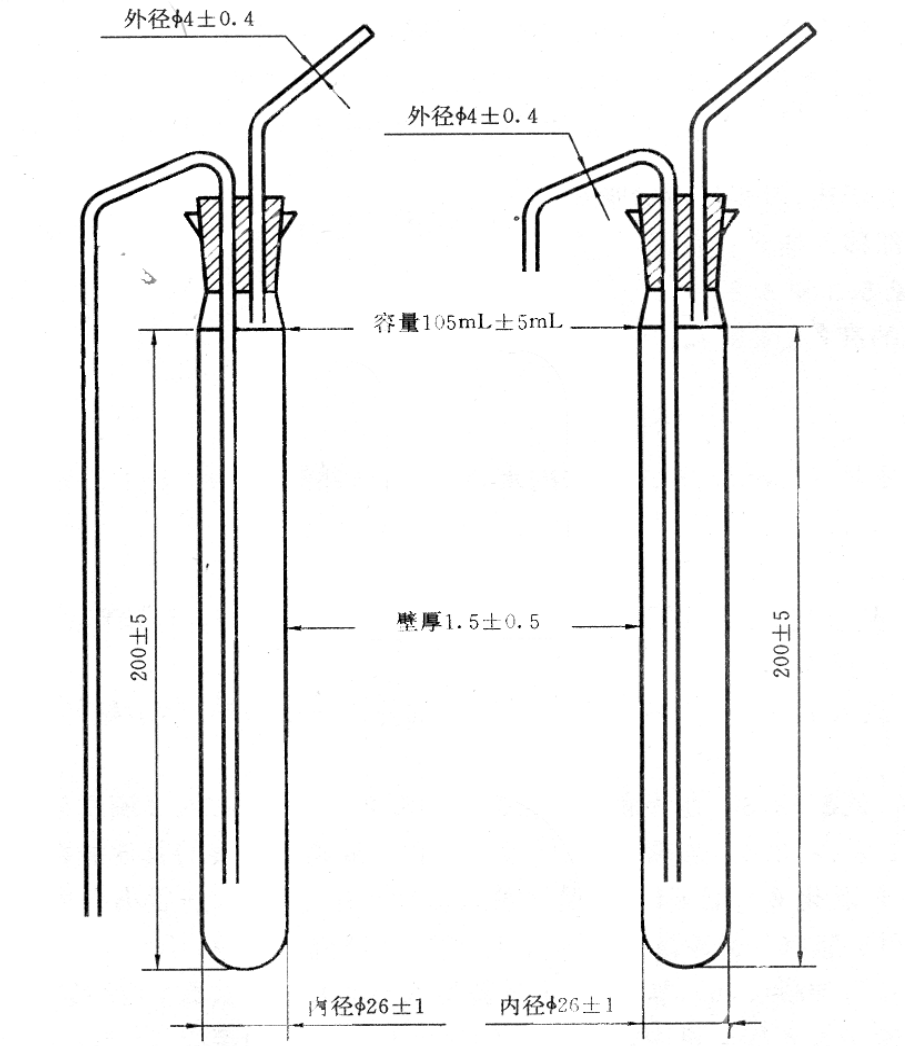
A.1.3.1.11 Repeat A.1.3.1.4 ~ A.1.3.1.9 for the second extraction but only with 15mL ethanol and 15mL petroleum ether. After extraction, flush the long branch pipe with ether when removing the pipe joint .

A.1.3.1.12 Repeat A.1.3.1.4 ~ A.1.3.1.9 for third extraction without ethanol and only 15ml ethanol and 15ml petroleum ether.

Note: If the fat content is less than 5%, a third extraction may be omitted.

A1.3.1.13 Follow 6.3.1.11 ~ 6.3.1.15 for below.

单位：mm



a）带虹吸管的抽脂管　　b）带洗瓶的抽脂管