

# National Standard of the People's Republic of China

GB 5009.6-2016

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## National food safety standard Determination of fat in foods

食品安全国家标准

食品中脂肪的测定

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## Foreword

This standard replaces GB/T 5009.6-2003 “Determination of fat in foods”, GB/T 9695.1-2008 “Meat and meat products Determination of free fat content”, GB 5413.3-2010 “National food safety standard Determination of fat in foods for infants and young children, milk and milk products”, GB/T 9695.7-2008 “Meat and meat products Determination of total fat content”, GB/T 14772-2008 “Determination of crude fat in foods”, GB/T 5512-2008 “Inspect of grain and oilseeds Determination of crude fat content in grain”, GB/T 15674-2009 “Determination of crude fat in edible mushroom”, GB/T 22427.3-2008 “Starches, native or modified Determination of total fat content” and GB/T 10359-2008 “Oilseed meals Determination of oil content--Part 1:Extraction method with hexane(or light petroleum)”.

Compared with GB/T 5009.6-2003, major changes of this standard are as follows:

- the standard name has been revised to “National food safety standard Determination of fat in foods”;
- the acid hydrolysis and extraction procedures of meat products and starches have been revised;
- the alkaline hydrolysis method and Gerber method have been added.

# National food safety standard

## Determination of fat in foods

### 1 Scope

This standard specifies the determination method of fat content in foods.

Method I of this standard is applicable to the determination of free fat content in the foods of fruits, vegetables and their products, grain and grain products, meat and meat products, egg and egg products, fishery and fishery products, bakery products and candies, *etc.*

Method II of this standard is applicable to the determination of total content of free fat and combined fat in the foods of fruits, vegetables and their products, grain and grain products, meat and meat products, egg and egg products, fishery and fishery products, bakery products and candies, *etc.*

Method III of this standard is applicable to the determination of fat in the milk, dairy products and formula foods for infants and young children.

Method IV of this standard is applicable to the determination of fat in the milk, dairy products and formula foods for infants and young children.

### Method I Soxhlet extraction method

#### 2 Principles

Fat is freely soluble in organic solvents. Extract test sample by absolute ether or petroleum ether directly, evaporate and remove solvent and dry, and free fat content is obtained.

#### 3 Reagents and Materials

Unless otherwise specified, all the reagents used in this method are analytically pure and water is Grade III water specified in GB/T 6682.

##### 3.1 Reagents

3.1.1 Absolute ether ( $C_4H_{10}O$ ).

3.1.2 Petroleum ether ( $C_nH_{2n+2}$ ): boiling range of petroleum ether is 30 °C~60 °C.

##### 3.2 Materials

3.2.1 Quartz sand.

3.2.2 Degreasing cotton.

#### 4 Apparatus and Equipment

4.1 Soxhlet extractor.

4.2 Constant temperature water bath.

4.3 Analytical balance: with sensitivity of 0.001 g and 0.000 1 g.

4.4 Electrothermal blowing dry oven.

4.5 Dryer: with effective drying agent inside (*e.g.* silica gel).

4.6 Filtration paper cylinder.

4.7 Evaporating dish.

#### 5 Analytical Procedures

##### 5.1 Test sample treatment

**5.1.1** Solid test sample: weigh 2 g~5 g (accurate to 0.001 g) of completely mixed test sample and transfer all the test sample into the filtration paper cylinder.

**5.1.2** Liquid or semi-solid test sample: weigh 5 g~10 g (accurate to 0.001 g) of completely mixed test sample, place in an evaporating dish, add about 20 g of quartz sand, evaporate to dry on the boiling water bath, dry in the electrothermal blowing dry oven at 100 °C±5 °C for 30 min, take out, grind, and transfer all the test sample into the filtration paper cylinder. Wipe the evaporating dish and glass rod with test sample using the degreasing cotton with ether and put the cotton in the filtration paper cylinder.

## 5.2 Extraction

Place filtration paper cylinder in the extraction cylinder of Soxhlet extractor, connect with the receiving flask that has been dried to constant weight, add absolute ether or petroleum ether to the position of two thirds of the flask volume from upper end of condenser tube of extractor, and heat on the water bath, so that absolute ether or petroleum ether back flows and extracts continuously (6 times/h~8 times/h). Generally, extract for 6 h~ 10 h. Pick 1 drop of extracting solution with a ground glass rod when extraction is over and if there is no oil spot on the ground glass rod, it shows extraction is over when.

## 5.3 Weighing

Take the receiving flask down, recycle absolute ether or petroleum ether, evaporate to dry when there is 1 mL~2 mL solvent in the receiving flask, dry at 100 °C±5 °C for 1 h, put in the dryer to cool for 0.5 h and then weigh. Repeat the above operations until constant weight (until difference between two weighing results does not exceed 2 mg).

## 6 Expression of Analysis Result

The content of fat in the test sample shall be calculated in accordance with Formula (1):

$$X = \frac{m_1 - m_0}{m_2} \times 100 \dots\dots\dots (1)$$

Where,

X—the content of fat in the test sample, g/100 g;

$m_1$ —the mass of receiving flask and fat after constant weight, g;

$m_0$ —the mass of receiving flask, g;

$m_2$ —the mass of test sample, g;

100—the conversion coefficient.

The calculation result shall keep one decimal place.

## 7 Precision

Absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of arithmetic mean value.

## Method II Acid hydrolysis method

### 8 Principles

Strong acid must be used to free the combined fat in foods and the freed acid is freely soluble in organic solvents. Hydrolyze the test sample with hydrochloric acid, then extract by absolute ether or petroleum ether, remove solvent and obtain the total content of free fat and combined fat.

### 9 Reagents and Materials

Unless otherwise specified, all the reagents used in this method are analytically pure and water is Grade III water specified in GB/T 6682.

#### 9.1 Reagents

**9.1.1** Hydrochloric acid (HCl).

**9.1.2** Ethanol (C<sub>2</sub>H<sub>5</sub>OH).

**9.1.3** Absolute ether (C<sub>4</sub>H<sub>10</sub>O).

**9.1.4** Petroleum ether (C<sub>n</sub>H<sub>2n+2</sub>): boiling range of petroleum ether is 30 °C~60 °C.

**9.1.5** Iodine (I<sub>2</sub>).

**9.1.6** Potassium iodide (KI).

## **9.2 Reagent preparation**

**9.2.1** Hydrochloric acid solution (2 mol/L): pipette 50 mL of hydrochloric acid, add into 250 mL of water and mix well.

**9.2.2** Iodine solution (0.05 mol/L): weigh 6.5 g of iodine and 25 g of potassium iodide, dissolve with small amount of water and dilute to 1 L.

## **9.3 Materials**

**9.3.1** Blue litmus paper.

**9.3.2** Degreasing cotton.

**9.3.3** Filter paper: medium speed.

## **10 Apparatus and Equipment**

**10.1** Thermostat water bath.

**10.2** Electric hot plate: meet with high temperature of 200 °C.

**10.3** Conical flask.

**10.4** Analytical balance: with sensitivity of 0.1 g and 0.001 g.

**10.5** Electrothermal blowing dry oven.

## **11 Analytical Procedures**

### **11.1 Acid hydrolysis of test sample**

#### **11.1.1 Meat products**

Weigh 3 g~5 g (accurate to 0.001 g) of well mixed test sample, place into a 250-mL conical flask, add 50 mL of 2 mol/L hydrochloric acid solution and several glass beads, cover the watch glass, heat to slight boiling on the electric hot plate, maintain for 1 h, and rotate and shake once every 10 min. Take the conical flask down, add 150 mL of hot water, mix well and filter. Wash the conical flask and watch glass with hot water and filter the hot water together. Wash the precipitate with hot water to neutral (test with blue litmus paper and if it is neutral, the color of the paper does not change). Place the precipitate and filter paper on a big watch glass, dry in the drying oven at 100 °C±5 °C for 1 h and cool.

#### **11.1.2 Starches**

Weigh 25 g~50 g (accurate to 0.1 g) of well mixed test sample in accordance with estimated value of total fat content, pour into a beaker and add 100 mL of water. Add 100 mL of hydrochloric acid into 200 mL water slowly, heat this solution on the electric hot plate, add it into the sample solution after it is boiling, heat the mixed solution to boiling and maintain for 5 min. Take several drops of the mixed solution into a test tube after stopping heating, cool, then add 1 drop of iodine solution, and carry out next step if blue does not appear. Continue boiling the mixed solution if blue appears, inspect with the above method continuously until there is no starch in the mixed solution and then continue the next step.

Place the beaker with mixed solution in the water bath boiler (70 °C~80 °C) for 30 min, stir ceaselessly to ensure temperature is uniform and separate out fat. Filter the cooled mixed solution with filter paper, and take out the fat adhering to inner wall of beaker with dry filter paper sheet. Filter the water washing the

beaker to make sure quantification is accurate. Wash precipitate and dry filter paper sheet with water at room temperature until color of filtrate does not change when tested by blue litmus paper. Fold the filter paper and dry filter paper sheet containing precipitate, place on a big watch glass and dry in the electrothermal constant-temperature dry oven at  $100\text{ }^{\circ}\text{C}\pm 5\text{ }^{\circ}\text{C}$  for 1 h.

### **11.1.3 Other foods**

**11.1.3.1** Solid test sample: weigh about 2 g~5 g (accurate to 0.001 g) of test sample, place in a 50-mL test tube, add 8 mL of water, mix well and add 10 mL of hydrochloric acid. Put the test tube in the water bath at  $70\text{ }^{\circ}\text{C}\sim 80\text{ }^{\circ}\text{C}$ , and stir once every 5 min~10 min using glass rod until test sample is totally digested, about 40 min~50 min.

**11.1.3.2** Liquid test sample: weigh about 10 g of test sample (accurate to 0.001 g), place in a 50-mL test tube and add 10 mL of hydrochloric acid. Other operations are the same as those in section 11.1.3.1.

### **11.2 Extraction**

#### **11.2.1 Meat products and starches**

Put the dried test sample in the filtration paper cylinder and other extraction procedure is the same as that in section 5.2.

#### **11.2.2 Other foods**

Add 10 mL of ethanol in a test tube and mix. Cool, transfer the mixture in a 100-mL measuring cylinder with stopper, wash the test tube with 25 mL of absolute ether for several times and pour into the measuring cylinder together. Shake for 1 min with stopper after all the absolute ether is poured in the measuring cylinder, open the stopper, release gas, then plug it again, put standing for 12 min, open the stopper carefully, and wash the fat adhered to the stopper and mouth of measuring cylinder with ether. Stand for 10 min~20 min, pipette supernatant liquid and place into the conical flask that has been dried to constant weight after top liquid is clear, add 5 mL of absolute ether into the measuring cylinder with stopper, shake, pipette the top ether after standing and put in the original conical flask.

### **11.3 Weighing**

The same as section 5.3.

## **12 Expression of Analysis Result**

The same as section 6.

## **13 Precision**

Absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of arithmetic mean value.

## **Method III Alkaline hydrolysis method**

### **14 Principles**

Extract alkaline (ammonia water) hydrolysate of test sample with absolute ether or petroleum ether, remove solvent through distillation or evaporation and determine the mass of abstract dissolved in the solvent.

### **15 Reagents and Materials**

Unless otherwise specified, all the reagents used in this method are analytically pure and water is Grade III water specified in GB/T 6682.

#### **15.1 Reagents**

**15.1.1** Amylase: with enzyme activity  $\geq 1.5\text{ U/mg}$ .

**15.1.2** Ammonia water ( $\text{NH}_3\cdot\text{H}_2\text{O}$ ): with mass fraction about 25%.

**Note:** The ammonia water with higher concentration can also be used.

**15.1.3** Ethanol (C<sub>2</sub>H<sub>5</sub>OH): volume fraction is at least 95%.

**15.1.4** Absolute ether (C<sub>4</sub>H<sub>10</sub>O).

**15.1.5** Petroleum ether (C<sub>n</sub>H<sub>2n+2</sub>): boiling range of petroleum ether is 30 °C~60 °C.

**15.1.6** Congo red (C<sub>32</sub>H<sub>22</sub>N<sub>6</sub>Na<sub>2</sub>O<sub>6</sub>S<sub>2</sub>).

**15.1.7** Hydrochloric acid (HCl).

**15.1.8** Iodine (I<sub>2</sub>).

## **15.2 Reagent preparation**

**15.2.1** Mixed solvent: mix equal volume of isopyknic ether and petroleum ether, prepare just before use.

**15.2.2** Iodine solution (0.1 mol/L): weigh 12.7 g of iodine and 25 g of potassium iodide, dissolve with water and dilute to 1 L.

**15.2.3** Congo red solution: dissolve 1 g of Congo red in water and dilute to 100 mL.

**Note:** It can be used selectively. Congo red solution can make the interface of solvent and water phase clear and other solutions that can stain water phase and does not affect determination result can also be used.

**15.2.4** Hydrochloric acid solution (6 mol/L): measure 50 mL of hydrochloric acid, pour into 40 mL of water slowly, dilute to 100 mL and mix well.

## **16 Apparatus and Equipment**

**16.1** Analytical balance: with sensitivity of 0.000 1 g.

**16.2** Centrifugal machine: liposuction flask or pipe can be placed in it, rotate speed is 500 r/min~600 r/min, it can generate the 80 g~90 g of gravitational field at the outer end of liposuction flask.

**16.3** Electrothermal blowing dry oven.

**16.4** Thermostat water bath.

**16.5** Dryer: with effective drying agent inside (*e.g.* silica gel).

**16.6** Liposuction flask: there shall be a cork or other stopper (*e.g.* silica gel or teflon) that does not affect use of solvent for the liposuction flask. Dip the cork in the ether first, then put into the water at 60 °C or above for at least 15 min, and use after cooling. Dip in the water when it is not used and replace soaking water once a day.

**Note:** Liposuction pipe (or flask) with siphon or wash bottle can also be used, but the operating procedure is different. Refer to the provisions in Annex A. The lower end of long branch pipe inside the joint can be made spoon shape.

## **17 Analytical Procedures**

### **17.1 Alkaline hydrolysis of test sample**

#### **17.1.1 Pasteurized milk, sterilized milk, raw milk, fermented milk and modified milk**

Weigh 10 g (accurate to 0.000 1 g) of sufficiently mixed test sample and put in a liposuction flask. Add 2.0 mL of ammonia water, mix sufficiently, then put the liposuction flask in the water bath at 65 °C±5 °C immediately, heat for 15 min~20 min, take out and shake frequently. Take out, cool to room temperature and stand for 30 s.

#### **17.1.2 Milk powder and food for infants and young children**

Weigh well mixed test sample, about 1 g (accurate to 0.0001 g) of high fat milk powder, whole milk powder, sweetened whole milk powder and food for infants and young children, about 1.5 g (accurate to 0.0001 g) of dried skim milk powder, whey powder and buttermilk powder, and other operations are the same as those in section 17.1.1.

##### **17.1.2.1 Sample free of starch**

Add 10 mL of 65 °C±5 °C water, wash the test sample into the small ball of liposuction flask, mix sufficiently until test sample totally disperses and place into the flowing water to cool.

#### **17.1.2.2 Sample with starch**

Put the test sample in a liposuction flask, add about 0.1 g of amylase, mix well, and then add 8 mL~10 mL of 45 °C water. Please note that the liquid level shall not be too high. Cover the cork, stir, place in 65 °C ±5 °C water bath for 2 h, and shake and mix once every 10 min. Add 2 drops of iodine solution with the concentration of about 0.1 mol/L to test whether the starch is totally hydrolyzed. If blue does not appear, starch is totally hydrolyzed, or put the liposuction flask in the water bath again until blue does not appear. Cool liposuction flask to room temperature. Other operations are the same as those in section 17.1.1.

#### **17.1.3 Condensed milk**

Weigh about 3 g~5 g of condensed skimmed milk, condensed whole milk and partial condensed skimmed milk, and about 1.5 g (accurate to 0.000 1 g) of condensed high fat milk. Wash into the small ball of liposuction flask for several times with 10 mL of water and mix well sufficiently. Other operations are the same as those in section 17.1.1.

#### **17.1.4 Cream and single cream**

Put cream test sample in the warm water bath to dissolve and mix well first, weigh about 0.5 g (accurate to 0.0001 g) of test sample (about 1 g for single cream), put in the liposuction flask and add 8 mL~10 mL of about 45 °C water. Then, add 2 mL of ammonia water and mix well sufficiently. Other operations are the same as those in section 17.1.1.

#### **17.1.5 Cheese**

Weigh about 2 g (accurate to 0.0001 g) of test sample, put in the liposuction flask, add 10 mL of 6 mol/L hydrochloric acid, mix well, cover the cork, heat in the boiling water for 20 min~30 min, take out to cool to room temperature and stand for 30 s.

### **17.2 Extraction**

**17.2.1** Add 10 mL of ethanol, and mix gently and thoroughly to avoid liquid is too close to the bottle neck. Add 2 drops of Congo red solution if necessary.

**17.2.2** Add 25 mL of ether, plug the cork, keep the liposuction flask at the horizontal position, clamp the extension part of small ball upward to the shaker, vibrate for 1 min according to the frequency of 100 times/min and manual vibration method can also be used. However, pay attention to forming the lasting emulsified liquid. Open the cork carefully after liposuction flask cools, wash the cork and bottle neck with a little of mixed solvent, and flow the washing liquid into the liposuction flask.

**17.2.3** Add 25 mL of petroleum ether, plug the re-wetted cork and vibrate for 30 s as shown in section 17.2.2.

**17.2.4** Place the liposuction flask with stopper in the centrifugal machine, centrifuge for 5 min at 500 r/min~600 r/min, or stand the liposuction flask for at least 30 min until the supernatant is clear and separate obviously from water phase.

**17.2.5** Open the cork carefully, wash the cork and inner wall of bottle neck with a little of mixed solvent, and flow the washing liquid into the liposuction flask.

Add water slowing along with the edge of flask wall if interface of two phases is lower than the connection of small ball and flask body, to ensure the liquid level is higher than the connection of small ball and flask body [see Figure 1a)], so that it's easy to pour out.



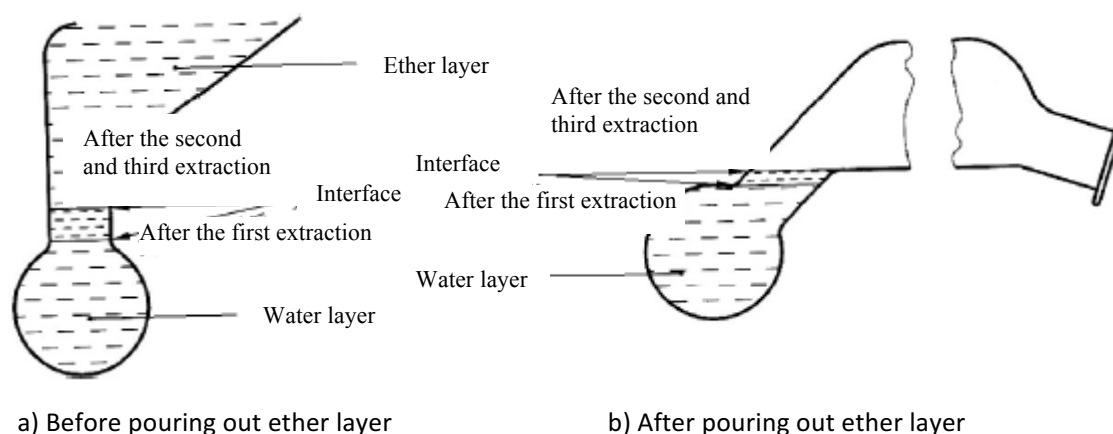


Figure 1 Operation diagram

**17.2.6** Pour the supernatant as much as possible into the prepared fat receiving flask with zeolite to avoid pour out the water layer [see Figure 1b)].

**17.2.7** Wash outside of the bottle neck with a little of mixed solvent and collect the washing liquid in the fat receiving flask. Prevent solvent splashing outside the liposuction flask.

**17.2.8** Add 5 mL of ethanol in the liposuction flask, wash inner wall of bottle neck with ethanol and mix in accordance with the procedure in section 17.2.1. Repeat operations in section 17.2.2~17.2.7, and carry out extraction for the second time with 15 mL of absolute ether and 15 mL of petroleum ether.

**17.2.9** Repeat operations in 17.2.2~17.2.7, and carry out extraction for the third time with 15 mL of absolute ether and 15 mL of petroleum ether.

**17.2.10** Conduct blank test simultaneously with sample inspection, replace test sample with 10 mL of water, carry out the same procedure and use the same reagents.

### 17.3 Weighing

Merge all the extracting solution and remove the solvent in the fat receiving flask can either distill, or evaporate to dry in the boiling water bath. Wash interior of bottle neck with a little of mixed solvent. Put fat receiving flask in the drying oven at  $100\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for 1 h, take out, cool in the dryer for 0.5 h and weigh. Repeat the above operations until it is constant weight (until difference between two weighing results does not exceed 2 mg).

## 18 Expression of Analysis Result

The content of fat in the test sample shall be calculated in accordance with Formula (2):

$$X = \frac{(m_1 - m_2) - ((m_3 - m_4))}{m} \times 100 \dots\dots\dots (2)$$

Where,

$X$ —the content of fat in the test sample, g/100 g;

$m_1$ —the mass of fat receiving flask and fat content after constant weight, g;

$m_2$ —the mass of receiving flask, g;

$m_3$ —the mass of fat receiving flask and abstract after constant weight in the blank test, g;

$m_4$ —the mass of receiving flask in the blank test, g;

$m$ —the mass of test sample, g;

100— the conversion coefficient.

The calculation result shall keep three decimal places.

## 19 Precision

Difference between two independent determination results is  $\leq 0.3$  g/100 g when fat content in the sample is  $\geq 15\%$ ;

Difference between two independent determination results is  $\leq 0.2$  g/100 g when fat content in the sample is between 5%~15%;

Difference between two independent determination results is  $\leq 0.1$  g/100 g when fat content in the sample is  $\leq 5\%$ .

## Method IV Gerber method

### 20 Principles

Add sulfuric acid into the milk to destroy emulsion property and the protein outer membrane covering on the fat ball, centrifuge fat and measure its volume.

### 21 Reagents and Materials

Unless otherwise specified, all the reagents used in this method are analytically pure and water is Grade III water specified in GB/T 6682

21.1 Sulfuric acid ( $H_2SO_4$ ).

21.2 Isopentanol ( $C_5H_{12}O$ ).

### 22 Apparatus and Equipment

22.1 Milk fat centrifugal machine.

22.2 Gerber milk fat meter: the minimum scale value is 0.1%, as shown in Figure 2.

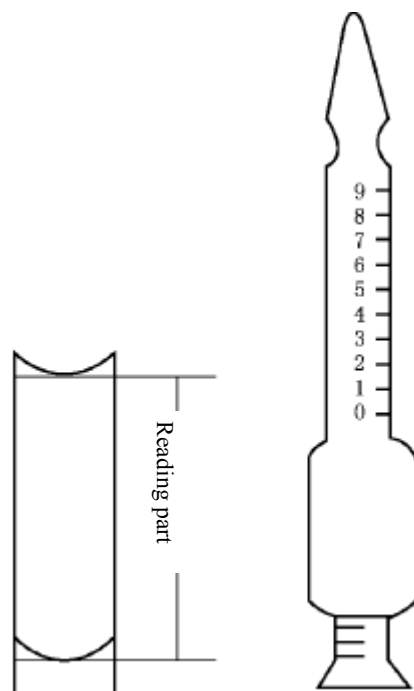


Figure 2 Gerber milk fat meter

22.3 10.75-mL single standard milk straw.

### 23 Analytical Procedures

Add 10 mL of sulfuric acid into Gerber milk fat meter first, then accurately add 10.75 mL of test sample carefully along with the pipe wall, ensure test sample doesn't mix with sulfuric acid, then add 1 mL of

isopentanol, plug the rubber stopper, put flask mouth downward, wrap with cloth in the meanwhile to prevent rushing out, vibrate violently to make the solution presents uniform brown, put standing for several minutes (flask mouth downward), place in the water bath at 65°C~70 °C for 5 min, take out, put in the milk fat centrifugal machine to centrifuge at 1100 r/min for 5 min, and then put in the water bath at 65°C~70 °C to keep warm for 5 min (pay attention to that water surface of water bath shall be higher than fat layer of milk fat meter). Take out, read immediately and it's the percentage of fat.

#### **24 Precision**

Absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 5% of arithmetic mean value.

## Annex A

### Operating steps of liposuction pipe with siphon or wash bottle

#### A.1 Alkaline Hydrolysis of Test Sample

##### A.1.1 Pasteurized milk, sterilized milk, raw milk, fermented milk and modified milk

Weigh 10 g (accurate to 0.001 g) of sufficiently mixed sample and put at the bottom of liposuction pipe. Add 2 mL of ammonia water and mix sufficiently with diluted sample at the bottom of pipe. Put liposuction pipe in the water bath at  $65\text{ }^{\circ}\text{C}\pm 5\text{ }^{\circ}\text{C}$  immediately, heat for 15 min~20 min, vibrate the sample pipe occasionally, and then cool to room temperature.

##### A.1.2 Milk powder and milk base food for infants and young children

Weigh well mixed sample, about 1 g of high fat milk powder, whole milk powder, sweetened whole milk powder and milk base food for infants and young children, about 1.5 g (accurate to 0.001 g) of dried skim milk powder, whey powder and buttermilk powder, put at the bottom of liposuction pipe, add 10 mL of water at  $65\text{ }^{\circ}\text{C}\pm 5\text{ }^{\circ}\text{C}$ , mix sufficiently until sample totally disperses and put in the flowing water to cool. Other operations are the same as those in A.1.1.

##### A.1.3 Condensed milk

Weigh about 10 g of condensed skimmed milk, 3 g~5 g of condensed whole milk and partial condensed skimmed milk, and about 1.5 g (accurate to 0.001 g) of condensed high fat milk and place at the bottom of liposuction pipe. Add 10 mL of water and mix well sufficiently. Other operations are the same as those in A.1.1.

##### A.1.4 Cream and single cream

Put cream sample in the warm water bath to dissolve and mix well first, weigh about 0.5 g of cream sample and about 1 g (accurate to 0.001 g) of single cream, put at the bottom of liposuction pipe. Other operations are the same as those in A.1.1.

##### A.1.5 Cheese

Weigh about 2 g (accurate to 0.001 g) of ground sample. Add 9 mL of water and 2 mL of ammonia water, stir with glass rod uniformly, heat slightly to dissolve casein, neutralize with hydrochloric acid, then add 10 mL of hydrochloric acid, add 0.5 g of sea sand, cover the glass lip, boil with gentle fire for 5 min, cool, transfer the content in the beaker at the bottom of liposuction pipe, wash beaker with 25 mL of absolute ether and put the washing liquid into the liposuction pipe.

#### A.2 Extraction

**A.2.1** Add 10 mL of absolute ethyl alcohol, gently mix sufficiently at the bottom of pipe and add two drops of Congo red solution when necessary.

**A.2.2** Add 25 mL of absolute ether, plug cork (water saturated) or other stoppers soaked in the water, reverse up and down for 1 min, but don't carry out overdue (avoid forming the lasting emulsified liquid). Put the pipe in the flowing water to cool when necessary, then carefully open the cork, wash the cork and pipe neck with a little of mixed solvent (use wash bottle) and flow the washing liquid into the pipe.

**A.2.3** Add 25 mL of petroleum ether, plug cork (re-wet the cork with water) and vibrate gently for 30 according to the operations in A.2.2.

**A.2.4** Put the pipe with cork in the centrifugal machine and centrifuge at 500 r/min~600 r/min for 1 min~5 min. Put standing at least 30 min until supernatant is clear and separates from water phase obviously, and cool.

**A.2.5** Open the cork carefully, wash the cork and pipe neck with a little of mixed solvent and flow the washing liquid into the pipe.

**A.2.6** Insert the siphon or wash bottle joint into the pipe, press the long branch pipe downward until it reaches to 4 mm above the interface of two phases and inside long branch pipe is parallel with the pipe axle.

Transfer the supernatant carefully into the fat receiving flask (or metal dish) with zeolite. Avoid transferring any water phase. Wash outlet of the long branch pipe with mixed solvent and collect the washing liquid into the fat receiving flask.

**A.2.7** Loosen the joint at the pipe neck, wash the joint and lower part of inside long branch pipe, re-insert the joint and transfer washing liquid into the fat receiving flask.

Wash outlet with a little of mixed solvent, put the washing liquid in the flask and remove part of solvent through distillation or evaporation according to operations in 17.3 when necessary.

**A.2.8** Loosen the joint at the pipe neck again, lift the joint slightly, add 5 mL of ethanol, wash the long branch pipe with ethanol and mix as shown in A.2.1.

**A.2.9** Repeat procedure in A.2.2~A.2.7 to carry out extraction for the second time, but only 15 mL of ether and 15 mL of petroleum ether are used. After extraction, wash the inside long branch pipe with ether when moving away pipe joint.

**A.2.10** Repeat procedure in A.2.2~A.2.7, not adding ethanol, carry out extraction for the third time, but only 15 mL of ether and 15 mL of petroleum ether are used.

**Note:** The third extraction can be omitted if mass fraction of fat in the product is lower than 5%.

**A.2.11** Carry out the following operations in accordance with 17.3.