Japan Customs Analysis Methods

No. 122

Analysis of Dairy Spreads

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1. Scope

This analysis method is applied to products which require to be confirmed whether their characteristics meet the "dairy spreads" stipulated in Note 2 (b) of Chapter 4 in the Customs Tariff Law (Appendix Table–Customs Tariff Schedule).

The emulsion type test of this analysis method is not applicable to products that are apparently inhomogeneous (i.e., not completely emulsified), such as those that separate into liquid and solid phases at 20ºC.

2. Outline of Test Method

This method confirms whether the sample of a dairy product meets the requirements of dairy spreads, according to the following procedure:

(1) Test for emulsion type (water-in-oil type) with lipophilic and hydrophilic reagents.
(2) Determination of fat extracted by acid digestion method with hydrochloric acid.
(3) Qualitative analysis of the fat by gas chromatography.

3. Apparatuses

(a) Extraction tube

Mojonnier tube described in "Standard Methods for the Analysis of Fats, Oils and Related Materials" or "Test Methods of Dairy Products".

(b) Gas chromatograph and columns

Gas chromatographs (with FID) capable of being equipped with the following columns:

(1) For qualitative analysis of triglyceride composition

Column (3 mmø × 20 cm) packed with DEXSIL 300GC 3% Chromosorb WAW DMCS 80/100 mesh or equivalent.

(2) For determination of fatty acid composition

Capillary column, e.g. DB-WAX (0.25 mmø × 30 m, 0.5 μm film thickness) or the like.

4. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

(a) Lipophilic reagent

Dissolve 10 mg of Sudan Blue (Oil Blue G Extra) in 1 mL of a mixture of soybean oil and heptane with a mixing ratio of 1:1[(1)].

(b) Hydrophilic reagent

Dissolve 10 mg of Acid Red 18 (newcoccin) in 1 mL of water.

(c) Petroleum ether

Boiling point should be in the range of 30–60ºC.

(d) Hydrochloric acid solution (specific gravity of about 1.125 / 20ºC)

Dilute 675 mL of conc.-hydrochloric acid (specific gravity of 1.18 / 20 ºC) with water to 1,000 mL.

(e) Mixed solvent

Prepare a mixture of diethyl ether and petroleum ether in equal quantities immediately before use.

(f) 0.5 mol/L sodium hydroxide-methanol solution

Dissolve 2 g of sodium hydroxide in 100 mL of

methanol.

(g) BF_3-methanol solution

Dissolve 125 g of BF_3 in 1,000 mL of methanol, or mix 10 mL of 40% BF_3 diethyl ether solution and 20 mL of methanol.

Note 1) If Sudan Blue (Oil Blue G Extra) is not dissolved completely and a portion of it remains as insoluble matter, use the supernatant.

5. Procedure

5.1. Test of emulsion type (water-in-oil type)

5.1.1. Preparation of samples

If a product to be analyzed is a rectangular solid, take an analysis sample with a thickness of less than 3 cm from it. Put it into a resealable plastic bag and seal after removing air from the bag as much as possible. Put the bag in another resealable plastic bag and seal it.

Sink the sealed sample entirely into a thermostat water bath at 20 ºC [(2)]. After leaving it for 2 hours, take the sample out from the bags.

Put the sample in a cup or a ring with a diameter of 2-3 cm and flatten the surface.

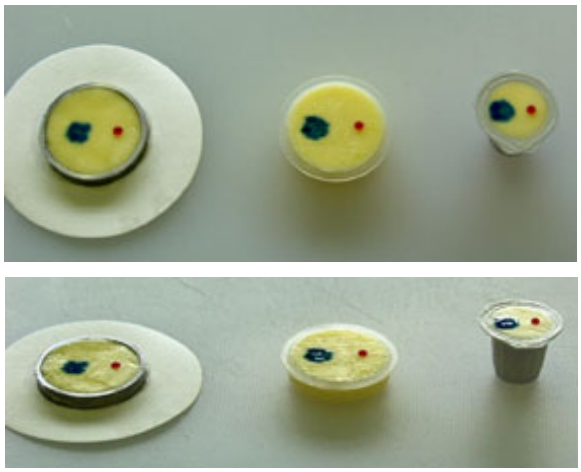
If surface flattening for the sample is difficult due to less ductility, conduct the test sample preparation above after putting the sample in the thermostat water bath for another 1 hour.

Note 2) Take care to avoid physical deformation of the sample.

5.1.2. Testing procedure

Drop 5 µL each of the lipophilic reagent and the hydrophilic reagent onto the test sample prepared in 5.1.1, and observe the affinity of the sample for each of the reagents. At the same time, perform the test for butter, which is a water-in-oil type, in the same manner as a reference and compare the behaviors of the sample and the butter to each of the reagents.

Samples showing its affinities for the lipophilic reagent but not for the hydrophilic reagent are identified as water-in-oil type substances by this test.



Test result examples: butter (water-in-oil type)

(Blue (left) and red (right) spots on test sample surfaces in cups or a ring are the lipophilic reagent and the hydrophilic reagent, respectively.)

5.2. Extraction and determination of fat content (hydrochloric acid digestion method)

5.2.1. Preparation of samples

In the case of frozen samples, return to a state at 20ºC in the same manner of 5.1.1 and homogenize by mixing.

5.2.2. Collection of samples

Accurately weigh about 0.5 g [(3)] of the original sample or sample prepared in 5.2.1 within an accuracy of 1 mg into a 100 mL beaker.

Note 3) Weigh the sample so that the amount of fat contained is 400-500 mg.

5.2.3. Measurement [(4)]

Add 8 mL of hydrochloric acid to the 100 mL beaker in which the sample has been put in 5.2.2, and disperse or dissolve particles completely by shaking it gently on a boiling water bath. Cover the beaker with a watch glass and leave it on a boiling water bath for 20-30 min. After cooling, transfer the content to an extraction tube. Wash the beaker with 10 mL of ethanol and transfer all washing to the extraction tube. Mix the contents in the extraction tube gently and sufficiently. [(5)]

Wash the beaker with 25 mL of diethyl ether and transfer all the washing into the extraction tube above. Seal it tightly with a stopper moistened with water (or a cork soaked with water). Shake it horizontally for a

minute. [6] Cool the extraction tube with running water if necessary. Remove the stopper (or the cork) from the extraction tube with care. Rinse the stopper and inner wall of the neck of the extraction tube with mixed solvent and put the washing in the extraction tube.

Wash the sample beaker with 25 mL of petroleum ether in the same manner and transfer the washings into the extraction tube above. Seal the extraction tube with the stopper (or cork) mentioned above and shake it gently for 30 seconds.

Leave the extraction tube with the stopper for at least 30 min so that the upper layer becomes transparent and separates clearly from the water layer. If necessary, cool it with running water.

Remove the stopper (or cork) with care. Rinse the stopper and the inner wall of the neck of the tube with a little amount of the mixed solvent, and add the washing into the extraction tube.

After allowing it to separate into layers, transfer as much of the upper layer solution as possible into an Erlenmeyer flask which has previously been weighed [7] by decantation with care while holding the small sphere of the extraction tube.

Add sequentially 15 mL of diethyl ether and 15 mL of petroleum ether to the residual in the extraction tube to extract again with the same procedure as above. At this time, rinse the inner wall of the neck of the extraction tube when adding diethyl ether.

Repeat the extraction with the same procedure as the second extraction, by adding 15 mL of diethyl ether and 15 mL of petroleum ether.

Completely evaporate the solvent (including ethanol) from the Erlenmeyer flask in a boiling water bath. Heat the Erlenmeyer flask in a drying oven at 102±2 ºC for an hour. Weigh it after cooling. Repeat this procedure until a constant weight can be obtained.

Note 4) Rohrig tubes for milk fat extraction can be used instead of Mojonnier tubes as extraction tubes. However, the same extraction procedure should be applied by making reference to this method.

Note 5) Take care to avoid cases that the solution goes up to the neck of the extraction tube.

Note 6) Perform a horizontal shaking of the Mojonnier tube vigorously (take care to avoid forming an emulsion) with the small bulb facing upward so that the solution in the large bulb flows to the small bulb regularly.

Note 7) Put boiling-stones in a 200 mL Erlenmeyer flask, dry it in a drying oven at 102±2 ºC for an hour, and weigh it after cooling.

5.2.4. Calculation

$$\%, \text{Fat content} = \frac{M_1 - M_0}{\text{weight of sample (g)}} \times 100$$

Where—

M_0: constant weight of an Erlenmeyer flask (g)

M_1: constant weight of an Erlenmeyer flask after extraction (g)

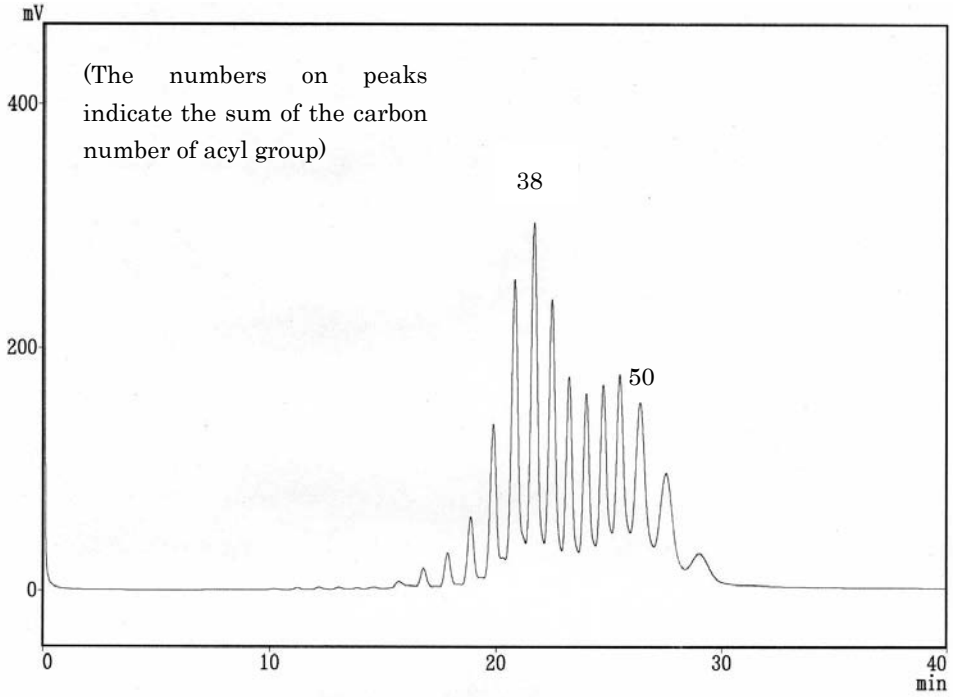
5.3 Qualitative analysis of milk fat by gas chromatography

5.3.1. Determination of triglyceride composition

Melt the fat extracted in 5.2 by heating and stir it uniformly. Weigh about 70 mg of it. Add 1 mL of chloroform and dissolve. Inject the solution directly into gas chromatograph. [8]

Identify the extracted fat by comparing the obtained gas chromatogram with that of standard milk fat measured under the same conditions.

(Reference) An example of gas chromatogram



Column:	glass column (3 mmø × 20 cm) packed with DEXSIL 300GC 2% Chromosorb WAW DMCS, 80/100 mesh.
Inlet and Detector Temp.:	injection port, 360 ºC; detector (FID), 360 ºC
Oven Temp.:	initial temp., 150 ºC for 5 min; ramp, 10 ºC/min; final temp., 350 ºC for 15 min
Carrier gas:	helium (constant pressure: 50 kPa)
Injection:	1 µL

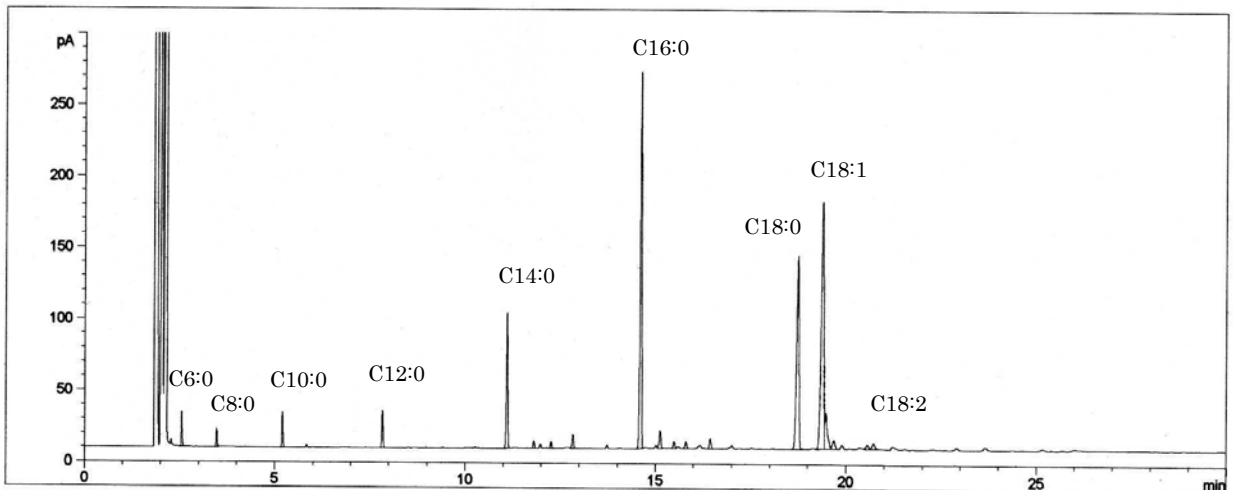
Note 8) GC separation conditions should be optimized for each type of gas chromatograph.

5.3.2. Determination of the fatty acid composition [9]

Melt fat extracted in 5.2 by heating and stirring it uniformly. Weigh about 200 mg of it into a 50 mL Erlenmeyer flask with a stopper. Add 4 mL of 0.5 mol/L sodium hydroxide-methanol solution and dissolve. After adding boiling-stones, heat it with a reflux condenser on a boiling water bath until the solution becomes uniform without any oil drop (normally 5-10 min). Add 5 mL of BF_3-methanol solution from the top of the condenser and continue boiling for 2 min. Add 2-5 mL of hexane from the top of the condenser and boil it for another 1 min. Terminate heating and remove the condenser. Add about 15 mL of saturated sodium chloride aqueous solution. Shake it vigorously for 15 seconds with a stopper and leave it until it becomes room temperature. Add another saturated sodium chloride aqueous solution until the hexane layer goes up to the neck of the flask. Transfer about 1 mL of the hexane layer from the flask to a test tube. Add a little amount of anhydrous sodium sulfate for dehydration, and inject it directly into gas chromatograph. [8]

Identify the extracted fat by comparing the obtained gas chromatogram with that of standard milk fat esterified and measured under the same conditions.

(Reference) An example of gas chromatograph



Column:	DB-WAX, 0.25 mmø × 30 m, 0.5 µm Film thickness
Inlet and Detector Temp.:	injection port, 230 ºC; detector (FID), 230 ºC
Oven Temp.:	initial 150 ºC for 1 min; ramp, 5 ºC/min; final 220 ºC for 15 min
Carrier gas:	helium (constant flow: 30 cm/sec)
Injection:	1 µL (split ratio, 25:1)

Note 9) Similar methyl esterification methods can also be applied.

6. References

(1) Koga T., Sasatani T. (2001) Report of the Central Customs Laboratory **41**: 23 (in Japanese).

(2) Takayama Y., Ebata K., Ikeda H., Setuda I., Ooki T. (2001) Report of the Central Customs Laboratory **41**: 17 (in Japanese).

(3) Test Methods of Dairy Products, Explanation (2nd revision), "1.3.2 Fat", ed. Pharmaceutical Society of Japan (1999) (in Japanese).

(4) AOAC 16th Edition 41.1.288 "Fatty acids in oils and fats" (1998)..

(5) Standard Methods for the Analysis of Fats, Oils and Related Materials or Test Methods of Dairy Products, "Ref. 3,1,3 acid decomposition method-1996", ed. Japan Oil Chemists' Society (in Japanese)

(6) Standard Methods for the Analysis of Fats, Oils and Related Materials or Test Methods of Dairy Products, "2.4.1.2 methyl esterification method-1996", ed. Japan Oil Chemists' Society (in Japanese).

(7) IDF STANDARD 5B CHEESE AND PROCESSED CHEESE PRODUCTS, "Determination of Fat Content" (1986).