

First Commission Directive of 13 November 1979 laying down  
Community methods of analysis for testing certain partly or wholly  
dehydrated preserved milk for human consumption (79/1067/EEC)

- Article 1 Member States shall take all measures necessary to ensure that...  
Article 2 Where alternative methods for a single determination are  
specified, the...  
Article 3 Member States shall bring into force the laws, regulations and...  
Article 4 This Directive is addressed to the Member States.  
Signature

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ANNEX I

SCOPE OF THE FIRST COMMUNITY METHODS OF ANALYSIS FOR CERTAIN  
PARTLY OR . WHOLLY DEHYDRATED PRESERVED MILK DIRECTIVE

- I. General provisions
- II. Determination of dry matter in:
- III. Determination of moisture in:
- IV. Determination of fat in:
- V. Determination of sucrose in:
- VI. Determination of lactic acid and lactates in:
- VII. Determination of phosphatase activity in:

ANNEX II

METHODS OF ANALYSIS RELATING TO THE COMPOSITION  
OF CERTAIN PARTLY OR WHOLLY DEHYDRATED PRESERVED  
MILK PRODUCTS INTENDED FOR HUMAN CONSUMPTION

GENERAL PROVISIONS

- 1. PREPARATION OF THE SAMPLE FOR CHEMICAL ANALYSIS
  - 1.1. Unsweetened condensed high fat milk
  - 1.2. Sweetened condensed milk
  - 1.3. Dried high fat milk or high fat milk powder
- 2. REAGENTS
  - 2.1. Water
    - 2.1.1. Wherever mention is made of water for dissolution, dilution or...
    - 2.1.2. Wherever reference is made to 'dissolution', 'solution' or 'dilution' without...

- 2.2. Chemicals
- 3. EQUIPMENT
  - 3.1. Lists of equipment
  - 3.2. Analytical balance
- 4. EXPRESSION OF RESULTS
  - 4.1. Calculation of percentage
  - 4.2. Number of significant figures
- 5. TEST REPORT

#### METHOD 1979/11/EEC DETERMINATION OF DRY MATTER CONTENT

- 1. SCOPE AND FIELD OF APPLICATION
- 2. DEFINITION
- 3. PRINCIPLE
- 4. REAGENTS
- 5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Metal dishes, preferably of nickel, aluminium or stainless steel. The...
  - 5.3. Atmospheric-pressure drying oven, well ventilated, thermostatically controlled with temperature regulated...
  - 5.4. Desiccator, containing freshly activated silica gel with a water content...
  - 5.5. Glass rods, flattened at one end of such a length...
  - 5.6. Waterbath, boiling.
- 6. PROCEDURE
  - 6.1. Place about 25 g sand (4) and a short glass...
  - 6.2. Without covering the dish and contents with the lid, place...
  - 6.3. Replace lid and transfer the dish to the desiccator (5.4)...
  - 6.4. Tilt the sand to one side of the dish. Introduce...
  - 6.5. Remove the lid, add 5 ml of water and, with...
  - 6.6. Place the dish on a boiling waterbath (5.6) until the...
  - 6.7. Place the dish and lid in the oven for one...
  - 6.8. Replace the lid, transfer the dish to the desiccator (5.4),...
  - 6.9. Replace the dish and lid in the oven, uncover the...
  - 6.10. Repeat process 6.8.
  - 6.11. Repeat the described processes 6.9 and 6.10 until the difference...
- 7. EXPRESSION OF RESULTS
  - 7.1. Method of calculation
  - 7.2. Repeatability
- 8. CALCULATION OF TOTAL MILK SOLIDS AND MILK SOLIDS NOT FAT...
  - 8.1. The total milk solids content of the sweetened condensed milk...
  - 8.2. The milk solids not fat content of the sweetened condensed...
  - 8.3. The milk solids not fat content of unsweetened condensed milks...

#### METHOD 1979/11/EEC DETERMINATION OF MOISTURE

- 1. SCOPE AND FIELD OF APPLICATION
- 2. DEFINITION
- 3. PRINCIPLE
- 4. APPARATUS
  - 4.1. Analytical balance.
  - 4.2. Dishes, preferably of nickel, aluminium, stainless steel or glass. The...
  - 4.3. Atmospheric-pressure drying oven, well ventilated, thermostatically controlled with temperature regulation...

- 4.4. Desiccator, containing freshly activated silica gel with a water content...
5. PROCEDURE
  - 5.1. Uncover the dish (4.2) and place it and its lid...
  - 5.2. Place the lid on the dish and transfer the covered...
  - 5.3. Introduce approximately 2 g of dried milk sample into the...
  - 5.4. Uncover the dish and put it with its lid in...
  - 5.5. Replace the lid, transfer the covered dish to the desiccator,...
  - 5.6. Uncover the dish and heat it and its lid for...
  - 5.7. Repeat process 5.5.
  - 5.8. Repeat processes 5.6 and 5.5 until the decrease in mass...
6. EXPRESSION OF RESULTS
  - 6.1. Method of calculation
  - 6.2. Repeatability

### METHOD 3: DETERMINATION OF FAT CONTENT IN CONDENSED MILKS (RÖSE-GOTTLIEB...

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Ammonia solution, approximately 25 % (m/m) NH<sub>3</sub> (density at 20 oC approximately...
  - 4.2. Ethanol, 96 ± 2 % (v/v) or, if not available, ethanol...
  - 4.3. Diethyl ether, peroxide-free.
  - 4.4. Light petroleum (petroleum ether), with any boiling range between 30...
  - 4.5. Mixed solvent, prepared shortly before use by mixing equal volume...
5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Suitable extraction tubes or flasks, provided with ground glass stoppers...
  - 5.3. Flasks, thin-walled and flat-bottomed, 150 to 250 ml capacity.
  - 5.4. Atmospheric pressure drying oven, well ventilated and thermostatically controlled (adjusted...
  - 5.5. Anti-bumping granules, fat-free, non porous, non friable in use, e.g....
  - 5.6. Siphon, to fit extraction tubes.
  - 5.7. Centrifuge (optional).
6. PROCEDURE
  - 6.1. Blank test
  - 6.2. Determination
    - 6.2.1. Dry a flask (5.3) (together with, if required, some anti-bumping...
    - 6.2.2. Stir the prepared sample and immediately weigh, to the nearest...
    - 6.2.3. Add 1,5 ml ammonia (25 %) (4.1) or a corresponding volume...
    - 6.2.4. Add 10 ml ethanol (4.2) and mix the liquids gently...
    - 6.2.5. Add 25 ml diethyl ether (4.3). Cool under running water....
    - 6.2.6. Remove the stopper carefully and add 25 ml light petroleum...
    - 6.2.7. Allow the apparatus to stand until the upper liquid layer...
    - 6.2.8. Remove the stopper, rinse it and the inside of the...
    - 6.2.9. Rinse the outside and the inside of the neck of...
    - 6.2.10. Make a second extraction by repeating the procedure of 6.2.5...

- 6.2.11. Make a third extraction by repeating the procedure of 6.2.10...
- 6.2.12. Carefully evaporate or distil off as much solvent (including the...
- 6.2.13. When there is no appreciable odour of solvent place the...
- 6.2.14. Remove the flask from the oven, allow to cool to...
- 6.2.15. Repeat 6.2.13 and 6.2.14 for heating periods of 30 to...
- 6.2.16. Add 15 to 25 ml light petroleum in order to...
- 6.2.16.1 If the extracted matter is wholly soluble in the light...
- 6.2.16.2 If any insoluble matter is present, or in case of...
- 7. EXPRESSION OF RESULTS
  - 7.1. Calculation
  - 7.2. Repeatability

#### METHOD 4: DETERMINATION OF FAT CONTENT IN DRIED MILKS (RÖSE-GOTTLIEB...

- 1. SCOPE AND FIELD AND APPLICATION
- 2. DEFINITION
- 3. PRINCIPLE
- 4. REAGENTS
  - 4.1. Ammonia solution, approximately 25 % (m/m) NH<sub>3</sub> (density at 20 °C approximately...
  - 4.2. Ethanol, 96 ± 2 % (v/v) or, if not available, ethanol...
  - 4.3. Diethyl ether, peroxide-free
  - 4.4. Light petroleum (petroleum ether), with any boiling range between 30...
  - 4.5. Mixed solvent, prepared shortly before use by mixing equal volumes...
- 5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Suitable extraction tubes or flasks, provided with ground glass stoppers...
  - 5.3. Flasks, thin-walled, flat-bottomed, of 150 to 250 ml capacity.
  - 5.4. Atmospheric pressure drying oven, well ventilated and thermostatically controlled (adjusted...
  - 5.5. Anti-bumping granules, fat-free, non porous, non friable in use, e.g....
  - 5.6. Waterbath, at 60 to 70 °C.
  - 5.7. Siphon to fit extraction tubes.
  - 5.8. Centrifuge (optional).
- 6. PROCEDURE
  - 6.1. Blank test
  - 6.2. Determination
    - 6.2.1. Dry the flask (5.3) together with, if required, some anti-bumping...
    - 6.2.2. Accurately weigh, to the nearest 1 mg, directly in, or...
    - 6.2.3. Add 1.5 ml ammonia (25 %) (4.1) or a corresponding volume...
    - 6.2.4. Add 10 ml ethanol (4.2) and mix the liquids gently...
    - 6.2.5. Add 25 ml diethyl ether (4.3). Cool in running water...
    - 6.2.6. Remove the stopper carefully and add 25 ml light petroleum...
    - 6.2.7. Allow the apparatus to stand until the upper liquid layer...
    - 6.2.8. Remove the stopper, rinse it and the inside of the...
    - 6.2.9. Rinse the outside and the inside of the neck of...
    - 6.2.10. Make a second extraction by repeating the procedure of 6.2.5...
    - 6.2.11. Make a third extraction by repeating the procedure of 6.2.10...

- 6.2.12. Carefully evaporate or distil off as much solvent (including the...
- 6.2.13. When there is no appreciable odour of solvent, place the...
- 6.2.14. Remove the flask from the oven, allow to cool to...
- 6.2.15. Repeat 6.2.13 and 6.2.14 for heating periods of 30 to...
- 6.2.16. Add 15 to 25 ml light petroleum in order to...
- 6.2.16.1 If the extracted matter is wholly soluble in the light...
- 6.2.16.2 If any insoluble matter is present, or in case of...
- 7. EXPRESSION OF RESULTS
  - 7.1. Calculation
  - 7.2. Repeatability

#### METHOD 5: DETERMINATION OF SUCROSE CONTENT (POLARIMETERIC METHOD)

- 1. SCOPE AND FIELD OF APPLICATION
- 2. DEFINITION
- 3. PRINCIPLE
- 4. REAGENTS
  - 4.1. Zinc acetate solution, 1 M: dissolve 21,9 g crystallized zinc...
  - 4.2. Potassium hexacyanoferrate (II) solution, 0,25 M: dissolve 10,6 g crystallized...
  - 4.3. Hydrochloric acid solution,  $6,35 \pm 0,2$  M (20 to 22 %)...
  - 4.4. Ammonia solution,  $2,0 \pm 0,2$  M (3,5 %).
  - 4.5. Acetic acid solution,  $2,0 \pm 0,2$  M (12 %).
  - 4.6. Bromothymol blue indicator, 1 % (m/v) solution in ethanol.
- 5. APPARATUS
  - 5.1. Balance, sensitivity 10 mg.
  - 5.2. Polarimeter tube, 2dm, of exactly calibrated length.
  - 5.3. Polarimeter or saccarimeter:
  - 5.4. Water bath, regulated at  $60 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ .
- 6. PROCEDURE
  - 6.1. Control determination
  - 6.2. Determination
    - 6.2.1. Weigh to within 10 mg, approximately 40 g of the...
    - 6.2.2. Transfer the mixture quantitatively to a 200 ml measuring flask,...
    - 6.2.3. Add 5 ml of the dilute ammonia solution (4.4). Mix...
    - 6.2.4. Neutralize the ammonia by adding an equivalent quantity of the...
    - 6.2.5. Add, with gently mixing by rotating the tilted flask, 12.5...
    - 6.2.6. Add 12.5 ml of potassium hexacyanoferrate (II) solution (4.2) in...
    - 6.2.7. Bring the contents of the flask to  $20 \text{ }^{\circ}\text{C}$  and make...
    - 6.2.8. Close the flask with a dry stopper and mix thoroughly...
    - 6.2.9. Allow to stand for a few minutes and then filter...
    - 6.2.10. Direct polarization: determine the optical rotation of the filtrate at...
    - 6.2.11. Inversion: pipette 40 ml of the filtrate obtained above into...
    - 6.2.12. Invert polarization
- 7. EXPRESSION OF RESULTS
  - 7.1. Method of calculation
  - 7.2. Values of the inversion factor Q
  - 7.3. Repeatability

**METHOD 6: DETERMINATION OF LACTIC ACID AND LACTATES CONTENT**

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Copper (II) sulphate solution: dissolve 250 g of copper (II)...
  - 4.2. Calcium hydroxide suspension.
    - 4.2.1. Grind 300 g of calcium hydroxide (Ca(OH)<sub>2</sub>) in a mortar...
    - 4.2.2. Calcium hydroxide suspension: grind 300 g of calcium hydroxide (Ca(OH)<sub>2</sub>)...
  - 4.3. Sulphuric acid — copper (II) sulphate solution: Add to 300...
  - 4.4. p-hydroxydiphenyl (C<sub>6</sub>H<sub>5</sub>C<sub>6</sub>H<sub>4</sub>OH) solution: dissolve, by shaking and by heating slightly...
  - 4.5. Lactic acid standard solution: dissolve, shortly before use, 0,1067 g...
  - 4.6. Standard reconstituted milk: analyse in advance several samples of high...
5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Spectrophotometer suitable for readings at a wavelength of 570 nm....
  - 5.3. Waterbath at 30 °C ± 2 °C.
  - 5.4. Mortar and pestle.
  - 5.5. Filter paper (Schleicher and Schull 595, Whatman 1 or equivalent)....
  - 5.6. Test tubes, pyrex or equivalent (dimensions 25 x 150 mm)....
6. PROCEDURE
  - 6.1. Blank test
  - 6.2. Determination
    - 6.2.1. Determine the solids-non-fat content (a) g of the sample by...
    - 6.2.2. Weigh 1000 a-10 g of the sample to the nearest...
    - 6.2.3. Pipette 5 ml of the solution obtained into a 50...
    - 6.2.4. Add slowly while shaking, 5 ml of the copper (II)...
    - 6.2.5. Add slowly while shaking, 5 ml of the calcium hydroxide...
    - 6.2.6. Dilute to 50 ml with water, shake vigorously, allow to...
    - 6.2.7. Pipette 1 ml of the filtrate into a test tube...
    - 6.2.8. Add to the tube by means of a burette or...
    - 6.2.9. Heat in the boiling water bath for five minutes. Cool...
    - 6.2.10. Add two drops of p-hydroxydiphenyl reagent (4.4) and shake vigorously...
    - 6.2.11. Place the tube in the boiling waterbath for 90 seconds....
    - 6.2.12. Measure the optical density against the blank test (6.1) within...
    - 6.2.13. If the optical density exceeds that of the highest point...
  - 6.3. Preparation of the standard
    - 6.3.1. Pipette 5 ml of the reconstituted milk (4.6) into five...
    - 6.3.2. Dilute with water to about 30 ml and treat as...
    - 6.3.3. Measure the optical densities of the standards (6.3.1) against the...
7. EXPRESSION OF RESULTS
  - 7.1. Method of calculation
  - 7.2. Repeatability

**METHOD 7: DETERMINATION OF PHOSPHATASE ACTIVITY (MODIFIED SANDERS AND SAGER...**

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION

3. PRINCIPLE
4. REAGENTS
  - 4.1. Solution A
  - 4.2. Solution B:
  - 4.3. Solution C
    - 4.3.1. Dissolve 0,5 g of disodiumphenylphosphate ( $\text{Na}_2\text{C}_6\text{H}_5\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) in 4,5 ml of...
    - 4.3.2. Pipette 1 ml of this solution into a 100 ml...
  - 4.4. Solution D
  - 4.5. Solution E
  - 4.6. Colour dilution buffer
  - 4.7. Copper sulphate solution
  - 4.8. Phenol standard solution
  - 4.9. Boiled distilled water.
  - 4.10. n-Butanol.
5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Waterbath, thermostatically controlled at  $37 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ .
  - 5.3. Spectrophotometer suitable for readings at a wavelength of 610 nm....
  - 5.4. Filter paper (Schleicher and Schull 597, Whatman 42 or equivalent...
  - 5.5. Waterbath, boiling.
  - 5.6. Aluminium foil.
6. PROCEDURE
  - 6.1. Preparation of the sample
    - 6.1.1. Weigh, to within 0.1 g, 10 g of the sample...
  - 6.2. Determination
    - 6.2.1. Introduce in each of two test tubes 1 ml of...
    - 6.2.2. Heat one of the tubes in boiling water for two...
    - 6.2.3. Add 10 ml of Solution C (4.3.2). Mix and place...
    - 6.2.4. Incubate for 60 minutes in the waterbath shaking periodically.
    - 6.2.5. Transfer the tubes immediately to a boiling waterbath (5.5) and...
    - 6.2.6. Add 1 ml of Solution D (4.4), mix and filter...
    - 6.2.7. Put 5 ml of each filtrate into test tubes, add...
    - 6.2.8. Allow the colour to develop at room temperature for 30...
    - 6.2.9. Measure the optical density of the sample solution, against the...
    - 6.2.10. Repeat the determination if the optical density of the solution...
7. PREPARATION OF THE STANDARD CURVE
  - 7.1. Pipette into four 100 ml volumetric flasks, 1, 3, 5...
  - 7.2. Pipette 1 ml of water or 1 ml of each...
  - 7.3. Pipette successively into each test tube 1 ml of the...
  - 7.4. Leave the test tubes for 30 minutes at room temperature...
  - 7.5. Measure the absorbance of the solutions in each of the...
  - 7.6. Prepare the standard curve by plotting the absorbance values against...
8. EXPRESSION OF THE RESULTS
  - 8.1. Calculation
    - 8.1.1. Convert the figures as determined under 6.2.9 to  $\mu\text{g}$  of...
    - 8.1.2. Calculate the phosphatase activity expressed as  $\mu\text{g}$  of phenol per...
    - 8.1.3. If it was necessary to dilute as indicated under 6.2.10...
  - 8.2. Repeatability

**METHOD 8: DETERMINATION OF PHOSPHATASE ACTIVITY  
(ASCHAFFENBURG AND MÜLLEN PROCEDURE)...**

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Sodium carbonate-bicarbonate buffer solution.
  - 4.2. Buffer substrate.
  - 4.3. Clarification solutions.
    - 4.3.1. Zinc sulphate solution.
    - 4.3.2. Potassium hexacyanoferrate (II) solution.
5. APPARATUS
  - 5.1. Analytical balance.
  - 5.2. Waterbath, thermostatically controlled at  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .
  - 5.3. Comparator, with special disc containing standard colour glasses calibrated in...
6. PROCEDURE
  - 6.1. Preparation of sample
  - 6.2. Determination
    - 6.2.1. Pipette 15 ml of buffer substrate (4.2) into a clean,...
    - 6.2.2. At the same time, place in the water bath a...
    - 6.2.3. After two hours remove both tubes from the water bath,...
    - 6.2.4. Transfer the filtrate to a 25 mm cell and compare...
7. EXPRESSION OF RESULTS
  - 7.1. Calculation
  - 7.2. Repeatability



(1) OJ No L 24, 30. 1. 1976, p. 49.