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)

ANNEX II

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

METHOD 6: DETERMINATION OF LACTIC ACID AND LACTATES CONTENT

1. SCOPE AND FIELD OF APPLICATION

This method determines the lactic acid and lactates, expressed as lactic acid, contents of:

— dried high fat milk or high fat milk powder,

— dried whole milk or whole milk powder,

— dried partly skimmed milk or partly skimmed-milk powder,

— dried skimmed milk or skimmed-milk powder.

2. DEFINITION

Lactic acid and lactates content of dried milks: the lactic acid and lactates, expressed as lactic acid, contents as determined by the method specified.

3. PRINCIPLE

Fat, protein and lactose are simultaneously removed from a solution of the sample by addition of copper sulphate and calcium hydroxide followed by filtration.

The lactic acid and lactates in the filtrate are converted into acetaldehyde by concentrated sulphuric acid in the presence of copper II sulphate.

The lactic acid content is determined colorimetrically using p-hydroxydiphenyl.

The lactic acid and lactates content is expressed as mg of lactic acid per 100 g of solids-non-fat.

4. REAGENTS

4.1. Copper (II) sulphate solution: dissolve 250 g of copper (II) sulphate (CuSO4.5H2O) in water and dilute to 1 000 ml with water.

4.2. Calcium hydroxide suspension.

4.2.1. Grind 300 g of calcium hydroxide (Ca(OH)2) in a mortar with water, using totally 900 ml. The suspension should be freshly prepared before use.

4.2.2. Calcium hydroxide suspension: grind 300 g of calcium hydroxide (Ca(OH)2) in a mortar with water, using totally 1 400 ml. The suspension should be freshly prepared before use.

4.3. Sulphuric acid — copper (II) sulphate solution: Add to 300 ml of sulphuric acid, 95,9 to 97,0 % (m/m) of H2SO4, 0,5 ml of the copper (II) sulphate solution (4.1).

4.4. p-hydroxydiphenyl (C6H5C6H4OH) solution: dissolve, by shaking and by heating slightly 0,75 g of p-hydroxydiphenyl in 5 ml of an aqueous solution of sodium hydroxide, containing 5 g of NaOH per 100 ml. Dilute to 50 ml with water in a volumetric flask. Keep the solution in a brown coloured glass bottle in a dark and cool place. Do not use if the colour changes or tubidity occurs. The maximum shelf life is 72 hours.

4.5. Lactic acid standard solution: dissolve, shortly before use, 0,1067 g of lithium lactate (CH3 CHOHCOOLi) in water and dilute to 1 000 ml in a volumetric flask. 1 ml of this solution corresponds to 0,1 mg of lactic acid.

4.6. Standard reconstituted milk: analyse in advance several samples of high quality dried milk. For the preparation of the calibration curve select the sample having the lowest lactic acid content, containing not more than 30 mg of lactic acid per 100 g of solids-non-fat. Follow the operating procedure described under 6.2.1 and 6.2.2 below.

5. APPARATUS

5.1. Analytical balance.

5.2. Spectrophotometer suitable for readings at a wavelength of 570 nm.

5.3. Waterbath at 30 ^{o}C ± 2 ^{o}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).

Note:

All glassware must be perfectly clean and designated for use solely in this determination. Rinse glassware containing precipitate residues with concentrated hydrochloric acid before washing.

6. PROCEDURE

6.1. Blank test

Carry out a blank test by placing 30 ml of water into a 50 ml graduated tube and treating this tube as described under 6.2.4 to 6.2.11 inclusive. If the blank measured against water exceeds an equivalent of 20 mg of lactic acid per 100 g solids-non-fat, the reagents should be checked and the impure reagents or reagent should be replaced. Carry out the blank test at the same time as the analysis of the sample.

6.2. Determination

Note: Avoid contamination with impurities especially with saliva and sweat.

6.2.1. Determine the solids-non-fat content (a) g of the sample by subtracting the fat content (obtained by method 4) and the moisture content (obtained by method 2) from 100.

6.2.2. Weigh

$$\frac{1000}{(a-10)}$$

g of the sample to the nearest 0,1 g. Add this quantity of sample to 100 ml of

6.2.3. Pipette 5 ml of the solution obtained into a 50 ml graduated tube and dilute with water to about 30 ml.

6.2.4. Add slowly while shaking, 5 ml of the copper (II) sulphate solution (4.1) and allow to stand for 10 minutes.

6.2.5. Add slowly while shaking, 5 ml of the calcium hydroxide suspension (4.2.1) or 10 ml of the calcium hydroxide suspension (4.2.2).

6.2.6. Dilute to 50 ml with water, shake vigorously, allow to stand for 10 minutes then filter. Discard the first runnings.

6.2.7. Pipette 1 ml of the filtrate into a test tube (5.6).

6.2.8. Add to the tube by means of a burette or graduated pipette 6.0 ml of the sulphuric acid-copper (II) sulphate solution (4.3). Mix.

6.2.9. Heat in the boiling water bath for five minutes. Cool to ambient temperature under running water.

6.2.10. Add two drops of p-hydroxydiphenyl reagent (4.4) and shake vigorously to spread the reagent evenly throughout the liquid. Place the tube in the waterbath at 30 ^{o}C ± 2 ^{o}C; leave for 15 minutes shaking from time to time.

6.2.11. Place the tube in the boiling waterbath for 90 seconds. Cool to ambient temperature under running water.

6.2.12. Measure the optical density against the blank test (6.1) within three hours at the wavelength specified under 5.2.

6.2.13. If the optical density exceeds that of the highest point of the standard curve, repeat the test using an adequate dilution of the filtrate obtained under 6.2.6.

6.3. Preparation of the standard

6.3.1. Pipette 5 ml of the reconstituted milk (4.6) into five 50 ml graduated tubes. Pipette into these tubes 0, 1, 2, 3 and 4 ml respectively of the standard solution (4.5), so as to obtain a range of standards corresponding to 0, 20, 40, 60 and 80 mg of added lactic acid per 100 g of solids-non-fat, of the dried milk.

6.3.2. Dilute with water to about 30 ml and treat as described under 6.2.4 to 6.2.11.

6.3.3. Measure the optical densities of the standards (6.3.1) against the blank test (6.1) at the wavelength specified under 5.2. Plot in a diagram the optical densities against the quantities of lactic acid given under 6.3.1, i.e. 0 mg, 20 mg, 40 mg, 60 mg and 80 mg per 100 g of solids-non-fat. Draw the best fitting straight line through the points and prepare the standard curve by moving this line parallel to itself in such a way that it passes through the origin.

7. EXPRESSION OF RESULTS

7.1. Method of calculation

Convert the optical density measured under 6.2.12 or 6.2.13 into mg of lactic acid per 100 g of solids-non-fat in the sample by reference to the standard curve. Multiply this result by the dilution factor where the filtrate has been diluted according to 6.2.13.

7.2. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 8 mg of lactic acid per 100 g of solids-non-fat for contents up to 80 mg. For higher values, this difference may not exceed 10 % of the lowest value.