Australian Standard™

Methods of chemical and physical testing for the dairying industry

Method 8.6: Anhydrous milk fat—Determination of peroxide value

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-010, Chemical Analysis of Dairy Products, to supersede AS 2300.8.6—1983, *Methods of chemical and physical testing for the dairying industry*—Part 8.6: *Anhydrous milk fat—Determination of peroxide value*.

This Standard was prepared by the Australian members of the Joint Standards Australia/Standards New Zealand Committee FT-010. After consultation with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The Committee considered ISO 3976:1977, *Anhydrous milk fat—Determination of peroxide value (Reference method)* for adoption. It was highlighted that the apparatus specified for the method is not used in Australia and the calibration curves are different to the Australian method. The ISO approach is not used in Australia as such laboratories cannot comply with the ISO Standard. Due to the above reasons, the Committee did not recommend the adoption of the ISO Standard as the Australian Standard. Therefore, it was recommended to re-issue the Australian Standard without any modification.

METHOD

1 SCOPE

This Standard sets out a method for the determination of the peroxide value of anhydrous milk fat.

2 APPLICATION

The method is applicable to anhydrous milk fat having a peroxide value not in excess of 1.0 milligram-equivalents of oxygen per kilogram.

3 REFERENCED DOCUMENTS

The following document is referred to in this Standard:

AS		
2300	Methods of chemical and physical testing for the dairying industry	
2300.8.1	Part 8.1:	Anhydrous milk fat—General requirements

4 DEFINITION

For the purpose of this Standard, the definition below applies.

4.1 Peroxide value

The number of milligram-equivalents of oxygen per kilogram of anhydrous milk fat, determined in accordance with the procedure described herein.

NOTE: Owing to the different stoichiometries of the reactions involved, the peroxide value determined by this method is about twice the peroxide value determined by the iodometric method. The exact factor varies slightly depending on the composition of the peroxides reacting and the exact conditions of the test. However, the method has good reproducibility and is extremely useful in detecting the early stages of fat deterioration.

5 PRINCIPLE

Ferrous chloride and ammonium thiocyanate are added to a solution of the sample in a chloroform/methanol mixture. The amount of ferric thiocyanate complex produced after a fixed reaction time with the peroxides present in the sample is determined colorimetrically at 500 nm.

6 REAGENTS

The following reagents are required and shall be in accordance with AS 2300.8.1:

(a) *Chloroform/methanol solvent*. Mix 70 volumes of chloroform with 30 volumes of anhydrous methanol.

(b) *Ferrous chloride solution*. Dissolve approximately 0.4 g barium chloride ($BaCl_2.2H_2O$) in about 50 mL of water.

Dissolve approximately 0.5 g iron (II) sulphate ($FeSO_4.7H_2O$) in about 50 mL of water. Slowly pour the barium chloride solution, with constant stirring, into the iron (II) sulphate solution and add about 2 mL of 10 mol/L hydrochloric acid.

Allow the precipitate of barium sulphate to settle or centrifuge the mixture until the upper liquid layer is clear. Decant the clear solution into a brown bottle.

NOTE: This solution should be prepared in indirect, dimmed light. It is stable for one week when stored at 4°C.

(c) *Ammonium thiocyanate solution*. Dissolve approximately 30 g ammonium thiocyanate (NH_4SCN) in water and dilute to 100 mL. If the solution is not colourless, remove the colour by extracting the solution several times with 5 mL portions of iso-amyl alcohol (3-methyl-butan-1-ol).

(d) *Standard ferric chloride solution* (10 µg/mL Fe). Dissolve 0.500 g bright iron wire in about 50 mL of 10 mol/L hydrochloric acid and add 1 mL to 2 mL of about 30 percent (m/m) hydrogen peroxide solution. Remove excess hydrogen peroxide by boiling for 5 min, then cool to room temperature. Transfer quantitatively to a 500 mL volumetric flask and make up to the mark with water. Pipette 1 mL of this solution into a 100 mL volumetric flask and make up to the mark with chloroform/methanol solvent.

(e) *Hydrochloric acid solution*, approximately 0.2 mol/L. Dilute 2 mL of about 10 mol/L hydrochloric acid to 100 mL with water.

7 APPARATUS

The following apparatus is required and shall be in accordance with AS 2300.8.1:

(a) Burettes, 10 mL capacity, graduated at each 0.02 mL.

(b) Pipettes, 1 mL capacity, graduated at each 0.01 mL.

(c) Pipettes, 10 mL capacity, graduated at each 0.1 mL.

(d) Test tubes, 150/20 mm with ground glass stoppers.

(e) Photometer, suitable for measuring at a wavelength of 500 nm, with appropriate cuvettes of 10 mm light path.

Precautions shall be taken to ensure that all apparatus is free from iron, grease and soap residues.

8 PROCEDURE

8.1 Precautions

In order to eliminate lipid oxidation, observe the following precautions:

(a) Carry out the test as soon as the sample is melted.

(b) Avoid, where possible, exposure of the sample of anhydrous milk fat to light.

(c) Complete the procedure from Clause 8.2 (a) to (g) inclusive, within 10 min including the 5 min reaction time.

(d) Carry out the test in indirect light, subdued as much as practicable.

8.2 Method

The procedure shall be as follows:

(a) From a graduated pipette, transfer 9.4 mL of the chloroform/methanol solvent into a 150/20 mm test tube.

(b) Prepare the sample as described in AS 2300.8.1.

(c) Add approximately 0.5 g of sample by the following method:

  (i) Prewarm a 1 mL graduated pipette in a Bunsen flame, and then fill it with sample.

  (ii) Wipe the outside of the pipette clean and deliver 0.6 mL of sample into the test tube.

(d) Estimate the mass of sample taken in Step (c) to the nearest 1 mg (m) by delivering, in the same manner, a minimum of three 0.6 mL aliquots into a tared weighing bottle and taking the average mass.

(e) From a graduated 1 mL pipette, add 0.05 mL of ammonium thiocyanate solution.

(f) From another 1 mL graduated pipette, add 0.05 mL of ferrous chloride solution. Mix thoroughly and allow to stand.

(g) After 5 min, measure and record the absorbance at a wavelength of 500 nm against the chloroform/methanol solvent.

(h) Carry out a fat blank test using Steps 8.2 (a), (b), (c), (d), (e) and (g) without the addition of ferrous chloride (Step (f)).

(i) Carry out a reagent blank test using Steps 8.2 (a), (b), (e), (f) and (g) without the addition of fat (Steps (c) and (d)).

9 REFERENCE CURVE

The procedure to plot a reference curve shall be as follows:

(a) Transfer from the 10 mL burette to four test tubes, 0.5 mL, 1.0 mL, 1.5 mL and 2.0 mL respectively of the standard ferric chloride solution in order to obtain a series containing 5, 10, 15 and 20 µg of iron (III).