

## STANDARDS ASSOCIATION OF AUSTRALIA

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**Australian Standard**  
**METHODS OF CHEMICAL AND PHYSICAL TESTING FOR THE**  
**DAIRYING INDUSTRY**

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## PART 8—ANHYDROUS MILK FAT

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**AS 2300.8.6**  
**DETERMINATION OF PEROXIDE VALUE**

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**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 DEFINITION.** For the purpose of this standard, the following definition applies:

*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.

**4 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.

**5 REAGENTS.**

- (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 ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) in about 50 mL of water.  
Dissolve approximately 0.5 g iron (II) sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) 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 ( $\text{NH}_4\text{SCN}$ ) 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.

**6 APPARATUS.**

- (a) Burettes, 10 mL capacity, graduated at each 0.02 mL.  
(b) Pipettes, 1 mL capacity, graduated at each 0.01 mL.