

METHOD 16.0

Effective 1st January 2018

VOLATILE MUSTARD OIL

1. Scope and Field of Application

This method is for the determination of volatile mustard oil contained in cakes made from the *Brassica and Sinapis* species, and in feedingstuffs which contain cakes made from those species. The steam separated component is expressed as allyl isothiocyanate.

2. Principle

The sample is suspended in water. The volatile mustard oil is released by the action of enzymes, entrained by distillation with ethanol and collected in dilute ammonia. The solution is treated while warm with a given volume of silver nitrate solution, then cooled and filtered. The excess silver nitrate is titrated with a solution of ammonium thiocyanate.

3. Reagents

3.1 Anti-foam (e.g. silicone).

3.2 Ethanol, 96% (V/V).

3.3 Nitric acid (d=1.42g/ml).

3.4 White mustard (*Sinapis alba*).

3.5 Ammonia solution: prepare by diluting one volume of ammonia (d=0.88.g/ml) with two volumes of water.

3.6 Ammonium ferric sulphate, saturated solution.

3.7 Ammonium thiocyanate solution, 0.1N.

3.8 Silver nitrate solution, 0.1N.

4. Apparatus

4.1 Flat-bottomed 500ml flasks with ground-glass stoppers.

4.2 Distilling apparatus fitted with a condenser and a splash head.

5. Procedure

Weigh to the nearest 0.001g, approximately 10g of the prepared sample and place in a 500ml flat-bottomed flask (4.1) and add 2g of finely ground white mustard (3.4) (an enzyme source) and 200ml water at 20°C. Stopper the flask and keep at 20°C for approximately 2 hours, shaking frequently. Add 40ml ethanol (3.2) and one drop of anti-foam (3.1). Distil approximately 150ml and collect the distillate in a 500ml conical flask containing 20ml ammonia (3.5) making sure that the end of the condenser is immersed in the liquid. Add to the ammoniacal solution 50ml 0.1N silver nitrate solution, (3.8) (or more if necessary), place a small funnel over the neck of the flask and heat the mixture on a steam bath for one hour. Allow to cool, transfer to a 250ml graduated flask rinsing in with water, make up to the mark, mix and filter. To 100ml of the clear filtrate, add 5ml nitric acid (3.3) and approximately 5ml ammonium ferric sulphate solution (3.6). Titrate the excess silver nitrate with the 0.1N ammonium thiocyanate solution (3.7).

Carry out a blank test by applying the same procedure to 2g finely ground white mustard (3.4), omitting the sample.

6. Expression of the Results

Subtract the volume of 0.1N silver nitrate solution consumed in the blank test from that consumed by the sample. The value obtained gives the number of ml of 0.1N silver nitrate solution consumed by the mustard oil in the sample. 1ml of 0.1N $\text{AgNO}_3 \equiv 4.956\text{mg}$ of allyl isothiocyanate. Express the result as allyl isothiocyanate as a percentage of the sample.