DETERMINATION OF THIOBARBITURIC ACID (TBA) NUMBER

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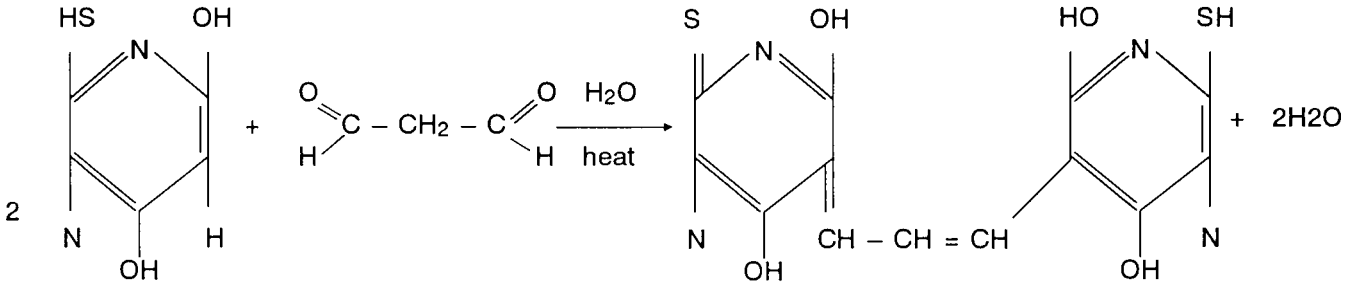
INTRODUCTION

In autoxidised lipids, most malonaldehyde does not appear in the free state but seems to exist mainly in a weakly-bound state and is released when the system is heated with a mild acid. The TBA test measures malonaldehyde in autoxidising systems. The basic reaction can be represented as follows:



Thiobarbituric acid

Malonaldehyde

Brilliant red product with absorption maximum at 532 nm

It is a sensitive test and can be correlated with the development of off-odours and flavours. It is especially well-suited for the detection of oxidative rancidity in lipids which are unsaturated and contain 3 or more double bonds. The TBA number is defined as the number of milligrammes of malonaldehyde per kilogramme of sample.

The results are expressed as malonaldehyde or 1,1,3,3,-tetra-ethoxypropane, which yields malonaldehyde by acid hydroloysis.

APPARATUS

1. Spectrophotometer (λ = 532 nm)
2. Test tubes with screw caps
3. Hot water bath (boiling water)
4. Pipettes (3, 5, 10, 25 ml)
5. Rotary evaporator with vacuum pump and water bath
6. Vortex mixer
7. Test tube basket
8. Glass centrifuge tubes
9. Centrifuge
10. Source of N_2 gas

REAGENTS

1. TBA solution

   Dissolve 1 g of TBA in 75 ml of 0.1N NaOH. Dilute to 100 ml with distilled water (can be kept for more than 1 month in refrigerator).

2. Trichloroacetic acid (TCA) solution

   Mix 50 ml of 25% TCA solution, 30 ml of 0.6 N HCl and 420 ml of distilled water.

3. Antioxidant solution

   Dissolve 0.3 g BHA (butylated hydroxyanisole) in 5.4 g propylene glycol. Dissolve 0.3 g of BHT (butylated hydroxytoluene) in 4.0 g of warm Tween 20. Mix the two solutions.

4. Chloroform (Analytical grade)

PROCEDURE

1. Take 0.2 - 0.4 g of fat sample or A ml of the extract containing 0.2 - 0.4 g of fat in a test tube with stoppers.
2. Add 3 drops of antioxidant solution.
3. Remove the solvent using the rotary evaporator under reduced pressure at 35 - 40°C (water-bath temperature).
4. Add 3 ml of TBA solution and 17 ml of TCA solution.
5. Flush N_2 gas into the test tube and immediately stopper.
6. Heat at 100°C in a boiling water-bath for 30 min till the colour appears.
7. Cool to room temperature in tap water.
8. Add about 5 ml of chloroform and mix for a few seconds with a Vortex mixer.
9. Transfer about 15 ml of the colour solution to a centrifuge tube.
10. Centrifuge for 10 min at 3,000 rpm.
11. If the aqueous solution is not clear, centrifuge again at 10,000 rpm for 10 min.
12. Transfer a part of the clear aqueous solution into another test tube and read absorbance at 532 nm.
13. Blank test should be carried out in the same manner without fats.

CALCULATION

$$\text{TBA No. (mg malonaldehyde/kg fat)} = \frac{\text{Abs.} \times F \times 0.2}{W}$$

where Abs = absorbance at 532 nm

W = weight of fat in volume of extract (g)

F = factor = 46

N.B The absorbance of a 1 g sample (in 100 ml reagent) multiplied by the factor 46 is the TBA number, or the milligram of malonaldehyde per 1000 g (1 kg) of sample (Sinnhuber et. al., 1958). As the amount of reagent used is only 20 ml, the results must be multiplied by 0.2 to give the absorbance of the sample in 100 ml reagent as specified by the definition.

REFERENCES

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