

Aim - Estimation of Thiobarbituric Acid (TBA) value

Thiobarbituric Acid Reactive Substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress. TBARS assay values are usually reported in malonaldehyde (malondialdehyde, MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides. Some of the aldehydes (malonaldehyde) of secondary oxidation products react with thiobarbituric acid, forming a reddish coloured product that can be determined spectrophotometrically. The results are expressed in terms of mg malonaldehyde present in one kg of fat. Malonaldehyde is produced due to methylene separated from fatty acid (dienoic or polyenoic) decomposition. TBA value is used for measuring rancidity in fatty fish.

Principle: The extraction of carbonyls and other thiobarbituric acid reactive substances is done under acidic condition. The thiobarbituric acid reacts with malonaldehyde to give a red chromogen, with absorbance maximum at 538nm.

Materials:

- TBA distillation unit
- 0.02M TBA reagent: 0.2883g thiobarbituric acid is dissolved in 100ml of 90% glacial acetic acid by slight warming the mixture in boiling water bath. The flask is wrapped in black foil and is prepared fresh daily.
- HCL solution: 1 part conc. HCL to 2 parts distilled water (approx. 4N)
- Spectrophotometer
- Glass test tubes
- Adjustable micropipettes (10 – 1,000 μ L) and tips 5

Method:

- Take accurately weigh 10g of sample and macerate with 47.5 ml distilled water to slurry using mortar and pestle.
- Transfer it to 500 ml round bottom flask.
- Add 2.5 ml of HCL solution to adjust the pH to 1.5.

- Flash the TBA distillation unit with steam and then place the sample flask.
- Discard the first few drops of distillate and then collect 50 ml distillate in a measuring cylinder within 10 min.
- Then take 5ml of distillate in a test tube and add 5 ml TBA reagent with it and mix well. Run a blank simultaneously with 5ml distilled water and 5 ml TBA reagent.
- Keep the tubes in a water bath for 30 min.
- Cool the tubes for 10 min and measure OD at 538nm.

Calculation:

TBA value (mg malonaldehyde per kg) =

Where,

A = Absorbance at 538nm

V = Volume of distillate taken

M = Mass of sample used