Aim 4

To Estimate the Sugar Content in the Biological sample by Anthrone method

Requirements

Apple, grapes, pomegranate, mango etc. are used as biological samples.

Reagents

1. Anthrone reagent (0.2 % in concentrated H_2SO_4)
2. Glucose (0.1 g/litre)

Procedure

1. Take 5.0 g of biological sample in a mortar.
2. Use pestle to homogenize with slowaddition of 25mlof 10mM phosphate buffer, pH 7.0.
3. Centrifuge the homogenous suspension.
4. Collect the supernatant, measure its volume (let Z ml) and calculate the sugar content after proper dilution (50x or 100x) depending upon the sugar content in the given sample.
5. Use anthrone method to determine the sugar content (As given in experiment - 3). In this case, take a control tube containing all the reagents,when the reaction stops, then add test sample while in the test sample tube, add test sample in the starting. Note down the readings of the test against control so that colour and non-specific reactions get subtracted.

Calculations

Let 1.0 ml of supernatant is diluted to 50ml (Solution A).

Then, 0.1ml (Solution A)is taken for estimation (suppose we get O.D -x).

0.1 ml of diluted supernatant (from solution A) contains y µg sugar.

So, 50ml will contain = (y/0.1)x 50µg sugar

Since we have diluted 1 ml supernatant to 50 ml

So 1 mlsupernatant contains = (50y/0.1)µg sugar

Z ml will contain = (50y/0.1)x Z µg sugar

Or (50y Z / 0.1x5) µg sugar/g sample

Or (10y Z/0.1) µg sugar/g sample

So, sugar content per g of a biological sample is 100yz µg.