Added sugar = Total sugars – Reducing sugars

(Ref :- A.O.A.C 17th edn, 2000 Official Method 925.35 Sucrose in Fruits and Fruit Products read with A.O.A.C Official method 923.09 Lane and Eynon general volumetric method)

*HPLC method can also be used for sugar profiling.

(Ref: - A.O.A.C 984.17: 'Method for the determination of Sugars in foods', *Jr. Agri. and Food Chemistry,* 19(3):551-54, (1971)(Modified) Brobst, K.M.

"Gas-Liquid Chromatography of Trimethylsilyl Derivatives, Methods in Carbohydrate Chemistry," 6:3-8, Academic Press, New York, NY,

(1972). (Modified))

2.8 Determination of Vitamin C (Ascorbic Acid):

The ascorbic acid content in fruits and vegetables can be estimated by macerating the sample with stabilising agents such as 20 % metaphosphoric acid.

2.8.1 Principle:

2, 6 -dichlorophenol indophenol is reduced to a colourless form by ascorbic acid. The reaction is specific for ascorbic acid at pH 1 to 3.5. The dye is blue in alkaline solution and pink in acid.

2.8.2 Reagents:

(1) Standard Indophenol Solution – Dissolve 0.05 gm 2, 6 dichlorophenol indophenol in 50 ml. water, to which 42 mg. sodium carbonate is added, and make upto 200 ml. with water and filter. Sodium carbonate is added for stability purpose. The dye solution keeps for a few weeks if stored in refrigerator. Prepare fresh if possible and standardize before use.

Blank correction: Dissolve 50 mg 2,6-dichloroindophenol Na salt that has been stored in desiccator over soda lime, in 50 mL H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye dis solves, dilute to 200 mL with H_2O. Filter through fluted paper into amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and

store in refrigerator. (Decomposition prod ucts that make end point in distinct occur in some batches of dry indophenol and also develop with time in stock solution. Add 5.0 mL extracting solution con taining excess ascorbic acid to 15 mL dye re agent. If reduced solution is not practically colorless, discard, and prepare new stock solution. If dry dye is at fault, obtain new supply.)

Transfer three 2.0 mL aliquots ascorbic acid standard solution to each of three 50 mL Erlenmeyers containing 5.0 mL HPO3–CH3COOH solution, B(a)(*1*). Titrate rapidly with indophenol solution from 50 mL burette until light but distinct rose pink persists ^{3}5 s. (Each titration should require ca 15 mL indophenol solution, and titrations should check within 0.1 mL).Similarly titrate 3 blanks com posed of 7.0 mL HPO3–CH3COOH solution, B(a)(*1*), plus volume H_2O ca equal to volume indophenol solution used in direct titrations. After substracting average blanks (usually ca 0.1 mL) from standardization titrations, calculate and express concentration of indophenol solution as mg ascorbic acid equivalent to 1.0 mL re agent. Standardize indophenol solution daily with freshly pre pared ascorbic acid standard solution.

(2) Standard Ascorbic acid solution – Dissolve 0.05 gm pure ascorbic acid in 60 ml of 20 % metaphosphoric acid (HPO3) and dilute with water to exactly 250 ml in a volumetric flask.

(3) Metaphosphoric acid - 20 %

(4) Acetone

2.8.3 Standardisation of Dye:

Pipette 10 ml of standard Ascorbic acid solution in a small flask and titrate with indophenol solution until a faint pink colour persists for 15 seconds. Express the concentration as mg Ascorbic acid equivalent to 1 ml of dye solution i.e 10 ml of

Ascorbic acid solution = 0.002 gm ascorbic acid

If 0.002 gm ascorbic acid requires V ml dye solution to neutralize it then 1 ml dye solution = 0.002 / V gm ascorbic acid.

2.8.4 Procedure

Pipette 50 ml of unconcentrated juice (or the equivalent of concentrated juice) into a 100 ml volumetric flask, add 25 ml of 20 % metaphosphoric acid as stabilizing agent and dilute to volume. Pipette 10 ml in a small flask and add 2.5 ml acetone. Titrate with indophenol solution until a faint pink colour persists for 15 seconds.

2.8.5 Calculation

Vitamin of Vitamin C per 100g/ml = Titer value x Dye factor X Vol made up X 100

Where, Aliquot x : is wt. or vol. of sample

mg Ascorbic acid /g, tablet, ml, etc. = (X – B) x (F/E) x (V/Y)

Where, X = average ml for test solution titration,

B = average ml for test blank titration,

F = mg ascorbic acid equivalent to one ml iodophenol standard solution,

E = no. of g, tablets, ml, etc. assayed

V= volume initial test solution and

Y= volume test solution titrated

Note:-

Acetone may be omitted if sulphur dioxide is known to be absent. Its function is to form the acetone bisulphate complex with sulphur dioxide which otherwise interferes with the titration. Sometime a small proportion of the ascorbic acid in foods becomes reversibly oxidized during aging and forms dehydroascorbic acid. If this is suspected, first estimate the ascorbic acid as above, then through another portion of the solution pass a stream of Hydrogen sulphide for 10 minutes. Stopper the flask and allow it to

stand overnight in a refrigerator. Then remove hydrogen sulphide by bubbling nitrogen through the mixture and titrated as before. The difference between the two titrations gives a measure of the dehydroascorbic acid. One international unit of vitamin C = 50 µg ascorbic acid.

(Ref :- F.A.O Manuals of Food Quality Control 14 / 8, page 194 / Pearson's Composition and Analysis of Foods 9th edn,1991, page 264 and AOAC Official Method 967.21 Ascorbic acid in Vitamin preparation and juices)

2.9 Determination of Ethanol Content

2.9.1 Principle

Note:

- This test method covers only the product which does not contain ethanol as an ingredient.
- The method is not applicable to products containing more than 5 % *(m/m)* of ethanol.

Separation of ethanol by distillation followed by oxidation by Potassium dichromate in a sulphuric acid medium and determination of excess dichromate by Ferrous ammonium sulphate in the presence of Ferrous 1, 10 phenathroline as indicator

2.9.2 Reagents

a) Concentrated Sulphuric acid – 1.84 gm / ml.
b) Dilute sulphuric acid –1.49 gm / ml (1+ 1).
c) Calcium hydroxide suspension obtained by shaking 110 – 112 gm of Calcium oxide in 1 litre water.
d) Potassium Dichromate solution containing 42.572 gm of $K_2Cr_2O_7$ per litre. 1 ml of this solution is equivalent to 0.01 gm ethanol.
e) Potassium Permanganate solution containing 1.372 gm of KMn O 4 per litre.