UNIT 15 TESTING OF FOOD INGREDIENTS

Structure

15.0 OBJECTIVES

After reading this unit, we will be able to :

- check the purity of food ingredients;
- know the presence and amount of different impurities in food ingredients;
- analyze different types of food ingredients;
- know about the apparatus and instruments used for the analysis of food ingredients ;
- prepare of different standard solutions and indicators used in titration;
- differentiate the food ingredients of same category; and
- Interpret the results obtained from the analysis.

15.1 INTRODUCTION

With the increased production of processed foods, manufacturers have started adding a large number of additives, generally in small quantities to improve the appearance, flavour, texture or shelf life of the processed foods. As certain impurities in these substances could be harmful, it is necessary to have a strict quality control of these food additives. BIS has prepared a series of specifications including methods for checking purity and identification of these additives. It is desirable to know about the conformity of additives to specifications before

intended use by manufacturers in specific processed foods. In this unit, the standard and simple protocol for checking the purity and method of analysis of impurities in food additives are discussed.

15.2 COLOURING MATTERS

Both types of colouring matter natural as well as synthetic are used in dairy products. Natural colours like b-carotene and annatto are preferred or synthetic colors. Methods, for testing of both types of colours are given below.

A. NATURAL COLOURING MATTERS

i) β-Carotene

Carotene is widely distributed in nature. It is an unsaturated hydrocarbon. It occurs in nature in three isomeric forms, a, â and g, out of which â-carotene is available in pure form and is also manufactured synthetically.

Assaying purity of â-carotene : Weigh accurately about 0.1 g of the dried sample and dissolve it in 100 ml of cyclohexane. Dilute the solution, stem-wise, to a final concentration of approx. 0.2 mg per 100 ml of cyclohexane. Measure the extinction of the resulting solution in a 1 cm cell at 456 nm.

Calculation

$$\text{Purity, \% by mass} = \frac{E \times 1000 \times 100}{2441 \times W}$$

Where

E = observed extinction, and

W= exact concentration of â-carotene in mg per 100 ml of cyclohexane in the final solution for extinction reading.

ii) Annatto Colour

Annatto colour is obtained from the aril (seed coat) of the plant *Bixa orellana.* Its solution in oil is used for colouring butter while its solution in water with a little alkali is used for colouring cheese and other similar products.

Assaying purity of Annatto

Annatto Extract in oil : Transfer 0.1 to 1 g of the sample, accurately weighed into a 100 ml volumetric flask. Dissolve in chloroform, dilute to volume with chloroform, and mix. Transfer a 1 ml portion of the solution into another 100 ml volumetric flask and dilute to 100 ml. Measure the absorbance A of this solution at 470 nm.

$$\text{Total carotenoids, \% by mass (expressed as bixin)} = \frac{A \times 1000 \times 100}{2.826 \times \text{mass of sample (mg)} \times 100}$$

Water Soluble Annatto : Weigh accurately 0.1 to 1 g of the sample, add 0.01N

sodium hydroxide to 100 ml, and shake thoroughly. Transfer 1 ml portion of the solution into a separating funnel, add 10 ml of sodium hydroxide solution (1 in 10) and water to 50 ml, and add 2 ml of diluted sulphuric acid. Continue to extract the solution with each 10 ml of benzene until the benzene extract is not coloured. Combine benzene extract, wash three times with each 5 ml of water, and allow to stand to separate the water layer. Transfer the benzene extract into another separating funnel containing the water layer three times with 2 ml of benzene, and combine the washings to the benzene extract. To the benzene extract, add an equal volume of petroleum benzene mix, and continue to extract with each 5 ml of 0.01N sodium hydroxide until sodium hydroxide solution is not coloured. Combine extracts, and add 0.01N sodium hydroxide to 100 ml. Measure the absorbance A at 453 nm.

$$\text{Total carotenoids, \% by mass (expressed as norbixin)} = \frac{A \times 1000 \times 100}{3.473 \times \text{mass of sample} \times 100}$$

iii) Saffron (Filaments And Powder Form)

Picrocrocine, Safranal & crocine

Weigh exactly 500 mg of sample and transfer quantitatively the test portion into the 1000 ml volumetric flask. Add about 900 ml of distilled water. Stir with a magnetic stirrer (1000 rpm) for 1 h, away from light. Remove the magnetic bar. Make up to the mark with distilled water. Close with a glass stopper and homogenize. Filter the solution, rapidly and away from light through the membrane so as to obtain a clear solution. Adjust the spectrophotometer and read the absorbance between 220 nm and 480 nm using distilled water as the reference liquid. The results are obtained by direct reading of the absorbance at 3 wavelengths, corresponding to the maximum absorbances of picrocrocine, safranal and crocine as follows:

Picrocrocine: absorbance at 257 nm

Safranal : absorbance at 330 nm

Crocine : absorbance at 440 nm

Identification of Saffron pigments

Reagents

a) Methanol

b) Ethanol

c) Chloroform

d) Sulphuric acid (95-97% m/m)

e) 4-Methoxybenzaldehyde (Anisaldehyde)

f) Naphthol yellow (Sodium salt of 2,4-dinitronaphthol)

g) Sudan red G

h) Reference solution prepared by dissolving 5 mg of Naphthol yellow in 5 ml of

methanol and adding a solution of 5 mg of Sudan red G in 5 ml of chloroform.

i) Elution solvent consisting of the organic phase of a mixture containing ethyl acetate : propan-2-ol : water (65:25:10)

j) Revealing solution prepared by mixing in the following order : 10 ml of anisaldehyde, 90 ml of ethanol and 10 ml sulphuric acid.

Apparatus

a) Chromatography chamber

b) Silica gel plates with indicator of fluorescence GF 254

Procedure

Weighing 0.05 g of sample into a test tube and moisten it with a drop of water. Wait for 2-3 min, and then add 1 ml of methanol. Allow the solution to settle for 29 min away from light. Filter it on a small glass wool plug. Using micropipette load 5 µl of solution on silica gel plate in 2 cm interval and develop in the chromatography chamber with the elution solvent until the solvent front has progressed 10 cm. Allow the solvent to evaporate. Examine the chromatogram in UV light at 254 nm, then in day light. Then spray onto the plate about 10 ml of the revealing solution. Heat for 5-10 min at a temperature of 105-110°C while observing the chromatogram.

Interpretation

1. In daylight the lower third of the chromatogram shows three yellow spots. The spot on the bottom strip is of more intense colour, and corresponds in colour and in size to the Naphthol yellow spot,it characterizes crocine.

2. The chromatogram observed in UV light at 254 nm shows 4 main fluorescent spots, 3 corresponding to the spots observed in daylight and another with a higher Rf value (»0.55), which characterizes picrocrocine. One or two rather faint spots of fluorescence are visible at the level of Sudan red G, characterizing â-hydroxycyclocitral and safranal.

3. After spraying with the revealing solution, the crocine becomes grayish green in colour, and the picrocrocine becomes violet in colour. The chromatogram shall not show any other colour spots before spraying, particularly at the starting point. These would correspond to a deterioration of the crocine and/ or the presence of foreign colouring matter.

iv. Caramel

Solid Content

The solid content of caramel colour is determined by drying a sample with acid washed sand (40-60 mesh). Mix 30 g of prepared sand accurately weighed with 1.5-2.0 g caramel colour accurately weighed and dry to constant weight at 60°C under reduced pressure 50 mm Hg. Record the final weight of the sand plus caramel. Calculate the percent solids as follows:

$$\text{Soilds content, \% by mass} = \frac{(WF - Wi) \times 100}{We}$$

Where,

Wf = final weight of sand plus caramel,

Ws= weight of sand, and

Wc= weight of caramel initially added.

Colour Intensity

Prepare a 0.1 percent m/v solution of the sample in freshly boiled and cooled distilled water. If the solution is not clear clarify it by centrifuging, not by filtering. Determine the absorbance of the clear solution in a 1 cm cell at 610 nm with a suitable spectrophotometer previously standardized using water as the reference. Calculate the colour intensity of the caramel colour as follows :

$$\text{Colour intensity} = \frac{A_{610} \times 100}{\text{Percent solids}}$$

Ammoniacal Nitrogen

Reagents

a) Sulphuric acid (0.1N)

b) Magnesium oxide (carbonate free)

c) Methyl red indicator solution (0.5% in ethanol)

d) Sodium hydroxide (0.1N)

Procedure

Add 25 ml of sulphuric acid to a 500 ml receiving flask and connect it to the distillation apparatus. Transfer about 20 g of the sample accurately weighed into a 800 ml long neck Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide, 200 ml of water and several boiling chips. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling and collect about 100 ml of distillate in the receiving flask. Wash the tip of the delivery tube with a few ml of water, collecting the washings in the receiving flask. Then add 4 to 5 drops of methyl red indicator and titrate with the sodium hydroxide, recording the volume (in ml) required as S. Conduct a blank determination and record volume (in ml) of the sodium hydroxide required as B.

$$\text{Ammoniacal } N_2\text{, \% by mass} = \frac{(S - B) \times 0.0014 \times 100}{\text{Mass of sample in g}}$$

v. 4-Methylimidazole

Caramel colour is added to a basic Celite column and eluted with mixture of chloroform and methanol. The eluent is extracted with dilute sulphuric acid and the aqueous extract concentrated, brought to a known volume and neutralized to

a slightly alkaline pH. The alkaline extract obtained is analyzed by paper chromatography. The developed spots are visualized by spraying with diazosulfanilic acid reagent, and sodium carbonate solution separately.

Reagents

a) Celite 545

b) Sodium hydroxide solution (2N)

c) Elution solvent (Chloroform:Ethanol=80:20, v/v)

d) Sulphuric acid solution (0.05N)

e) Developing solvent-1 containing diethyl ether, chloroform, methanol and ammonium hydroxide= 80:20:20:4(v/v)

f) Developing solvent-2 containing 3 parts of n-propyl alcohol and 1 part of 0.2N ammonia solution.

g) Spraying reagent-1 : (a) Prepare freshly a 5.0% m/m solution of sodium nitrite, cool to 0°C.

(b) Add 0.9 g of sulphanilic acid to 9.0 ml of conc. HCl taken in a 100 ml flask and dilute to 100 ml with distilled water. Cool to 0°C.

h) Spraying reagent-2 : 5 % m/v sodium carbonate solution.

i) Stock solution of 4MeI : Dissolve 100 mg of 4-methylimidazole in 100 ml of 0.1N sulphuric acid. Store the solution in a refrigerator.

j) Standard solution of 4-MeI: Pipette out 1 ml of 4-MeI stock solution in a 10 ml flask and dilute to volume with distilled water. Bring the pH of the solution to 9 using solid sodium carbonate. The solution corresponds to 100 mg/kg.

Procedure

Extraction: Prepare a basic column packing by mixing well Celite 545 and 2N NaOH in the proportion of 2 ml of 2N NaOH to 3 g Celite 545. Place a fine glass wool plug at the base of the chromatographic column, followed by 5.0 g of basic column packing. Tap the packing firmly to a uniform mass.

Take 10 g of sample of caramel in 150 ml beaker. To this add 6 g of 20 % Na_2CO_3 solution. Mix well and add 12.0 g Celite 545. After thoroughly mixing add the mixture to the prepared column. Dry, wash the beaker with about 2.0 g Celite 545 and add this wash to column. Place a plug a glass wool at the top of the dry wash and tap the contents of the column.

Elute the column with elution mixture until 125 ml has been collected. The elution rate should be 5 ml/min and collected into 250 ml separating funnel. Extract the elute with 20 ml portion of 0.05N sulphuric acid and then with second 10 ml portion of 0.05N sulphuric acid. Transfer the combined aqueous layers to 100 ml vacuum flask and concentrate to 5 ml by drying under vacuum at a temperature not exceeding 55°C (the volume should not be reduced below 4 ml)

Transfer this aqueous solution into a 10 ml volumetric flask, which also receives rinsing with 1 ml portion of distilled water until the mark is reached. After mixing transfer the aqueous concentrate to a suitable sample vial and treat with small portion of sodium carbonate anhydrous powder until CO_2 evolution ceases and pH meter indicates pH 9.

Paper chromatography: Spot 10 ml of this solution on the chromatographic paper 3 cm from the bottom of the paper, and develop the chromatogram at 25-28°C by ascending paper chromatography using either of the developing solvent. Allow the solvent to travel up to 15 cm.

Remove the paper from the tank and dry it by air drier. Spray the paper with diazosulfanilic acid reagent until the paper is just wet and then develop the colour by a light spray of 5% m/v sodium carbonate solution. Cut the maroon colour spots and elute in 20 % ethanol (5-10 ml). Keep aside for 30 min and immediately note the absorbance of solution at 505 nm. Side by side prepare the calibrated graph using various concentrations of 4-MeI and the amount of 4-MeI in the unknown is the red off directly from the above calibrated graph.

$$\text{Equivalent colour basis} = \frac{\text{Concentration on solids basis} \times 0.1}{\text{Colour intensity}}$$

This gives content expressed in terms of a product having a colour intensity of 0.1 absorbance units.

2-Acetyl-4-Tetrahydroxy Butylimidazole (Thi)

THI is converted into its 2,4-dinitrophenyl hydrazone. This derivative is separated from excess reagent and carbonylic contaminants by HPLC on RP-8, then determined its absorbance at 385 nm.

Reagents

Cation-exchange resin (strong): Dowex 50 AG×8, H+, 100-200 mesh

Cation-exchange resin (weak): Amberlite CG AG, H+, 100-200 mesh

Methanol (carbonyl free)

Dimethoxyethane

Procedure

Caramel colour (200-250 mg) is weighed accurately then dissolved in water (3 ml). The solution is passed through a combined column (upper column : weakly acidic cation exchanger, 50-60 mm bed height/ 12.5 mm i.d. or 80-90 mm bed height / 10 mm i.d. and lower column : strongly acidic cation exchanger, 60 mm bed height) with 100 ml of water. The upper column is disconnected. The lower column is eluted with 0.5M HCl. The first 10 ml of elute are discarded then a volume of 35 ml is collected. The solution is concentrated to dryness at 40°C and 15 torr. The syrup residue is dissolved in methanol (250 ml) and 2,4-dinitrophenyl hydrazine reagent (250 ml) is added. The reaction mixture is transferred to a septum-capped vial and stored for 5 h at room temperature.

A volume of 1-25 µl is injected on to a Lichrosorb RP-8 (10 mm) HPLC column.

The mobile phase is methanol/0.1M H_3PO_4 : 50:50 (v/v). At a mobile phase flow rate of 2 ml/min and column dimensions of 250 mm × 4.6 mm, THI-DNPH is eluted at about 6.3±0.1 min. It is detected at 385 nm and the peak height is measured. The amount is calculated from a calibration curve prepared with THI-DNPH in methanol.

Total Sulphur

Take a 100 ml crucible, place 1-3 g MgO, 1 g sucrose powder and 50 ml HNO_3. Add 5-10 g caramel colour. Place same quantity of reagents in another crucible as blank. Evaporate on steam bath to paste. Place in cold muffle furnace and gradually heat (525°C) until all NO_2 fumes are driven off. Cool, dissolve and neutralize with dilute HCL, adding excess of 5 ml. Filter, heat to boiling and add 5 ml of 10 % $BaCl_2.2H_2O$ solution dropwise. Evaporate to 100 ml, let stand overnight, filter, wash, ignite and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g.

Sulphur Dioxide

Reagents

a) Hydrogen peroxide (3% free from sulphate)

b) Barium chloride (10% m/v)

c) Hydrochloric acid (2N)

d) Conc. Hydrochloric acid

e) Carbon dioxide gas

f) Sodium hydroxide (0.1N)

g) Bromophenol blue indicator (Dissolve 0.5 g of bromophenol blue in 7.5 ml of 0.1N sodium hydroxide and dilute to 1 litre.

Procedure

Measure 25 ml of hydrogen peroxide into the conical flask and add 25 ml of distilled water. Measure 10 ml of hydrogen peroxide and add into the first Wohler tube (U tube). In the second tube (as a guard) place 5 ml of a mixture of equal volumes of hydrogen peroxide and barium chloride solutions which has been slightly acidified with 2N HCl solution.

Introduce 500 ml of distilled water and 20 ml of conc. HCl into the round bottom flask. Connect the CO_2 tube to the flask and boil the water for a short time in a current of CO_2 gas Introduce about 20 g of sample accurately weighed into the round bottom flask, through the funnel, continuing the flow of CO_2 gas. While adding the sample, see that there shall not be any back pressure. Boil it for 2h passing a slow current of CO_2 gas throughout the test. Before the end of the distillation stop the flow of water through the condenser to allow any sulphur dioxide which is retained by the condensed moisture in the tube of the condenser to be driven over into the receiver.

As soon as the delivery tube, condenser is hot, disconnect the straight tube first,

wash it with water. Combine the contents of first Wohler tube in the receiver or conical flask. Cool the conical flask and titrate with the NaOH solution using bromophenol blue indicator. Calculate the percentage of sulphur dioxide. 1 ml of 0.1N NaOH is equivalent to 3.203 mg of sulphur dioxide (Figure 15.1).

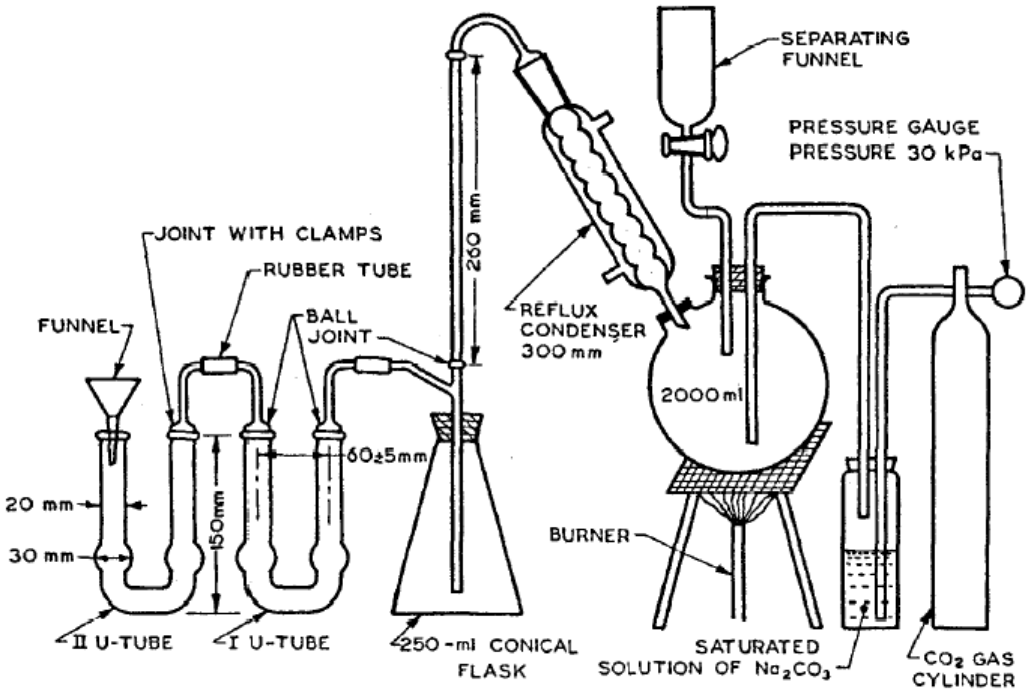

Figure 15.1

B. Permitted Synthetic Food Colours

i) Subsidiary Dyes

The subsidiary dyes are separated from the main dye by ascending paper chromatography and are extracted separately from the paper. The optical densities of the extracts are measured at their wavelengths of maximum absorption in the visible spectrum and are used to calculate the content of subsidiary dyes as a percentage by mass of the sample.

Reagents

(a) Chromatographic solvents

- Water:ammonia (sp gr. 0.880):trisodium citrate (95 ml:5 ml:2 g)
- n-Butanol:water:ethanol:ammonia (600:264:135:6)
- Butan-2-one:acetone:water (7:3:3)
- Butan-2-one:acetone:water:ammonia (700:300:300:2)
- Butan-2-one:acetone:water (700:160:300:2)
- n-Butanol:glacial acetic acid:water (4:1:5)

(b) Extracting solvent : A mixture of equal volumes of acetone and water.

(c) Sodium bicarbonate (0.05N)

Procedure

Not less than 2 h before carrying out the determination arrange the filter paper drapes in the glass tank and pour over the drapes and into the bottom of the tank sufficient of the atmosphere saturating solvent to cover the bottom of the tank to a depth of 1 cm.

Mark out a sheet of chromatography grade paper. Apply 0.1 ml of a 1 % aqueous solution of the dye a s uniformly as possible within the contents of the 180 mm ×7 mm rectangle, holding the nozzle of the micro-syringe steadily in contact with the paper. Allow the paper to dry at room temperature for 1-2 h or at 50°C for 5 min followed by 15 min at room temperature. Mount the sheet together with a plain sheet to act as a blank. Pour sufficient chromatography solvent into the tray to bring the surface of the solvent about 1 cm below the base line of the sheet of paper. Allow the solvent front to ascent the full height of paper, development being continued for 1 h afterwards, then remove the frame and transfer it to a drying cabinet at 50-60°C for 10-15 min. Remove the sheets from frame.

Cut each subsidiary band from the sheet as a strip and cut an equivalent strip from the corresponding position of the plain sheet. Place each strip into different test tubes. Add 5 ml of extracting solvent to each test tube, swirl for 2-3 min, add 15 ml of the sodium carbonate solution and shake the tube to ensure mixing. Filter the coloured extracts and blanks through a filter paper and determine wavelengths of maximum absorption against a filtered mixture of 5 ml of extracting solvent and 15 ml of sodium bicarbonate solution. Measure the optical density odf the extract of the blank strips at the wavelengths at which those of the corresponding coloured extracts were measured.

Subsidiary dye, % by mass = $F\ [(D_1+D_2+\ldots..)-(b_1+b_2+\ldots\ )]$

Where,

F= conversion factor (11.4),

D_1, D_2, etc = optical densities of the subsidiary dye extracts; and

b_1, b_2, etc. = optical densities of extracts of the corresponding blanks.

ii) Dye intermediates

Apparatus

Chromatography tube

Column preparation: Prepare a slurry of Whatman powdered cellulose in a 25 % ammonium sulphate solution. Prepare the column and pass 200 ml of 25% ammonium sulphate solution through it. The UV absorption of solution shall be sufficiently low to avoid interference with the intended analysis. Use about 75 g of cellulose to 500 ml of liquid. Pour sufficient slurry into the tube to give a column to a height of about 5 cm in the mouth of the tube. Tap the tube occasionally to ensure a well packed column. Wash the column with 200 ml of the eluent.

Procedure

Place 0.2 g of the dye sample in a beaker and dissolve in 20 ml of water. Add

5 g of powder cellulose. Add 50 g of ammonium sulphate to the dye. Transfer the mixture to the column, rinse the beaker with 25 % ammonium sulphate solution and add washings to the tube. Allow the column to drain until flow ceases or nearly so. Add the ammonium sulphate solution to the column at a rate equivalent to the rate of flow through the column. Collect the effluent in 100 ml fractions. Continue until 12 fractions have been collected. Mix each fraction well and obtain the UV absorption spectra of each solution from 220 to 400 nm. The specific spectra may be chosen depending on the nature of the dyes.

iii) Unsulphonated Primary Aromatic Amines

Unsulphonated primary aromatic amines are extracted into toluene from an alkaline solution of the sample, re-extracted into acid and then determined spectrophotometrically after diazotisation and coupling.

Procedure

Weigh about 2 g of the colour sample into a separating funnel containing 100 ml of water, swirl down the sides of the funnel with further 50 ml of water. Swirl to dissolve the sample, add 5 ml of 1N NaOH solution. Extract with two 50 ml portion of 0.1N NaOH solution to remove traces of colour. Extract the washed toluene with three 10 ml portions of 3N HCl solution and dilute the combine extract to 100 ml with water. Mix well. Pipette 10 ml of this solution into a clean, dry test tube, cool for 10 min by immersion in a beaker of ice/water mixture; add 1 ml of 50% KBr solution and 0.05 ml of 0.5N sodium nitrite solution. Mix and allow to stand for 10 min in the ice/water bath and add 1 ml of 0.05N disodium salt of 2-napthol-3,6-disulphonic acid (R-salt). Dilute to 25 ml with water, stopper flask and mix the content well and allow to stand for 15 min in the dark. Measure the absorbance of coupled solution at 510 nm in 1 cm cell using as a reference mixture of 10 ml of 1N HCl, 10 ml of 2N sodium carbonate solution and 2 ml of R-salt solution, diluted to 25 ml with water. Similarly, measure absorbances of 10 ml of aniline standard solutions containing 50 to 250 µg aniline, after diazotisation and coupling.

iv. Leuco Base

Air is blown through an aqueous solution containing the chloride and dimethylformamide. Under these conditions the leuco base is oxidized to colouring matters and the increase in absorptivity is a measure of the amount of leuco base originally present.

Reagents

- Solution A: Weigh 10 g of CuCl2.2H2O and dissolve in 200 ml of DMF. Transfer to a 1 litre volumetric flask and make up to mark with DMF.

- Solution B: Accurately weigh the specified quantity of sample, dissolve in 100 ml of water, transfer quantitatively to a 10 litre volumetric flask and make up to the mark with water.

Procedure

Prepare the following solutions:

Solution A-Puipette 50 ml DMF into a 250 ml volumetric flask. Cover with parafilm and place in the dark.

Solution B-Accurately pipette 10 ml of solution B into a 250 ml volumetric flask. Add 50 ml DMF. Cover with parafilm and place in the dark.

Solution C-Pipette 50 ml of solution A into a 250 ml volumetric flask. Bubble air through this solution for 30 min in the following manner.

Insert a 5 ml pipette into a box attached to a bench air flow source. Turn on the air, slowly. Stick the pipette down into the solution in the flask and adjust the air flow to a rapid but controlled rate. After 30 min pull the pipette out of the solution and rinse the sides of the pipette into the flask with water from a wash bottle. Then turn off the air flow.

Solution D-Accurately pipette 10 ml solution B into two separate 250 ml volumetric flasks in the same manner as used for solution B. Add 50 ml solution to each flask. Bubble air through the solutions for 30 min, using the above method.

After 30 min of rapid bubbling of air through the solutions, dilute all 5 flasks nearly to volume with water. Heat is evolved when DMF and water are mixed, so place the flask in a water bath of tap water until they have cooled to room temperature. Bring accurately to volume with water. Run the solutions on the spectrophotometer immediately. The entire procedure should be completed as quickly as possible.

Draw the following curves from 700-500 nm using an absorbance range of 0.1 and 1 cm cells.

Calculation

$$\text{Leuco base, \% by mass} = \frac{[(4-3)-(2-1)]\times 25\times 100}{A\times M\times \text{Ratio}}$$

Where,

A = absorptivity of 100% colouring matters,

M = mass, in g, of sample taken for test,

Ratio = MW of colouring matter/MW of leuco base

4 = Run curve without zero setting, record absorbance at maximum,

3 = Set zero at 700 nm, record absorbance at Abs std colouring matter,

2 = Run curve without readjusting zero setting , record absorbance at maximum,

1 = Set zero at 700 nm, record absorbance at Abs std for colouring matter.

v) Chlorides (As Sodium Chloride)

Accurately weigh 0.5-1.0 g of dye sample, dissolve n 100 ml of water, and acidify with 5 ml of 1.5N nitric acid solution. Place the silver electrode in the colour solution and connect the calomel electrode to the solution by means of the saturated potassium sulphate bridge. The saturated potassium sulphate bridge may be eliminated by using a glass electrode as the reference electrode. Determine the chloride content of the solution by titration against the 0.1N silver nitrate solution and calculate the result as sodium chloride. 1 ml of 0.1N silver nitrate is equivalent to 0.00585 g of sodium chloride.

vi) Metallic Impurities

The samples are dissolved in acid or digested in a mixture of sulphuric acid, nitric acid and in some cases perchloric acid. The barium, cadmium, lead, copper, chromium and zinc in solution are determined by flame atomic absorption spectroscopy. Antimony and arsenic are determined by using a hydride generation technique.

Procedure

Accurately weigh about 2.5 g of the sample into a 500 ml Kjeldahl flask, add 5 ml of dilute nitric acid. As soon as any initial reaction subsides, heat gently until further vigorous reaction ceases and then cool. Add gradually 4 ml of conc. sulphuric acid. at such a rate as not to cause excessive frothing on heating and then heat until the liquid darkens appreciably in colour, that is, begins to clear.

Add conc nitric acid slowly in small portions, heating between additions until darkening again takes place. Do not heat so strongly that charring is excessive; small but not excessive amount of free nitric acid should be present throughout. Continue this treatment until the solution is only pale yellow in colour and fails to darken in colour on prolonged heating. If the solution is still coloured run in 0.5 ml of hydrogen peroxide and heat further for a few minutes longer. Allow to cool somewhat and dilute with 10 ml of water. The solution should be quite colourless. Boil down gently, taking care to avoid bumping, until white fumes appear. Allow to cool, add a further 5 ml of water and again boil down gently to fuming. Finally cool and add 10 ml of 5N HCl and boil gently for a few min. Cool and transfer the solution to a 50 ml volumetric flask washing out the Kjeldahl flask with small portions of water and dilute to the mark with water. If barium is present, add 0.0954 g of KCl before dilution as an ionizing buffer to prevent ionization of barium.

Instrumental conditions

Select the wavelength and gases to be used for the particular element under consideration from the table below

Element	Wavelength (nm)	Gases
Antimony	217.6	Air/Acetylene
Barium	553.6	Nitrous oxide/acetylene
Cadmium	228.8	Air/Acetylene
Chromium	357.9	Nitrous oxide/acetylene
Copper	324.8	Air/Acetylene
Lead	283.3	Air/Acetylene
Zinc	213.9	Air/Acetylene

Set the AAS to the appropriate conditions. Aspirate the strongest standard containing the element to be determined and optimize the instrument settings to give maximum deflection. Plot a calibration curve with 3-4 standard solutions. Aspirate the test solution and the corresponding blank solution.

Calculation

$$\text{Element (X) in the sample, mg/kg} = \frac{\text{Conc of X in test solution} - \text{Conc of X in blank } (\mu g/ml) \times 50}{\text{Mass of sample taken (g)}}$$

Arsenic By Hydride Generation Technique

Arsenic is determined after preparation of their volatile hydrides mixing of 10% HCl and 10% sodium borohydride solution with sample solution, which are collected in the generation vessel. The gases are then expelled with argon or nitrogen gas into a hydrogen flame. The arsenic is then determined by using all other steps involved in conventional flame atomic absorption spectroscopy.

Mercury By Atomic Absorption Cold Vapour Technique

The sample is digested by heating under reflux with sulphuric and nitric acids. The oxidation is completed by addition of potassium permanganate solution. After successive additions of hydroxylamine hydrochloride solution and stannous chloride solution, the mercury content is measured by cold vapour atomic absorption spectrometry.

vii) Heavy Metals

Reagents

- Ammonia solution (Dilute 400 ml of ammonium hydroxide (28%) to 1 litre with water)
- Hydrochloric acid (10%)
- Lead nitrate stock solution (Dissolve 159.8 mg of lead nitrate in 100 ml of water containing 1 ml of nitric acid. Dilute with water to 1 litre and mix. Prepare and store the solution in lead free glass containers.
- Standard lead solution (Dilute 10 ml of lead nitrate stock solution, accurately measured with water to 100 ml. Each ml of the solution contains 10 mg of lead. Prepare the solution on the day of use.
- Nitric acid (10%)
- Concentrated Sulphuric acid
- Hydrogen sulphide (A saturated solution of hydrogen sulphide gas made by mixing of iron sulphide with dilute hydrochloric acid solution, which passing through cold water).

Procedure

Solution A : Take the quantity of standard lead solution of concentration equivalent to the limits specified in the individual Nessler tube and add about 23 ml of water. Adjust the pH to between 3-4 by addition of acetic acid or ammonia solution. Dilute with water to 40 ml and mix.

Solution B: Place 500 mg of the sample, accurately weighed, in a suitable crucible,

add sufficient sulphuric acid to wet the sample, carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulphuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in muffle furnace at 500-600°C until the carbon is completely burned off. Cool and add 4 ml of dilute HCl, cover and digest on a steam-bath to dryness. Moisten the residue with 1 drop of HCl, add 10 ml of hot water and digest for 2 min. Add dropwise ammonia solution until the solution is just alkaline to litmus paper dilute with water to 25 ml and adjust the pH to 3-4 by the addition of dilute acetic acid. Filter if necessary; wash the crucible and the filter with 10 ml of water. Transfer to a 50 ml Nessler tube. Dilute the combined filtrate and washing with water to 40 ml and mix.

To each tube add 10 ml of freshly prepared hydrogen sulphide, mix and allow to stand for 5 min and view over a white surface. The colour of solution B shall not be darker than of solution A.

viii) Ponceau 4r

Purity

Weigh accurately about 250 mg of the dye sample and dissolve in 0.1N HCl in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 1mg per 100 ml. Find out the optical density of this diluted solution against 0.1N HCl solution as blank at 506 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{\text{OD} \times 100}{\text{M} \times 440}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

440 = Extinction coefficient for Ponceau 4R in 0.1N HCl solution.

Subsidiary dye

The details of the method given above shall be followed.

Developing solvent No. 3

Height of ascent of solvent front= 17 cm then 1 h for further development.

ix. Carmoisine

Purity

Weigh accurately about 250 mg of the dye sample and dissolve in 0.1N HCl in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 1mg per 100 ml. Find out the optical density of this diluted solution against 0.1N HCl solution as blank at 516 nm in a glass cell with 10 mm

path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 520}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

520 = Extinction coefficient for carmoisine in 0.1N HCl solution.

Subsidiary dye

The details of the method given above shall be followed.

Developing solvent No. 4

Height of ascent of solvent front= 17 cm.

x. Erythrosine

Purity

Weigh accurately about 125 mg of the dye sample and dissolve in with 0.1N NaOH in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 0.5 mg per 100 ml. Find out the optical density of this diluted solution against 0.1N NaOH solution as blank at 527 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 1080}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

1080 = Extinction coefficient for erythrosine in 0.1N NaOH solution.

Subsidiary dye

The details of the method given above shall be followed.

Developing solvent No. 5

Height of ascent of solvent front= 17 cm.

Fluorescein

Solvent - methanol: water: ammonia = 500 :400:100

Sample – Weigh 1 g sample and dissolve in 50 ml solvent and dilute to 100 ml in a volumetric flask.

Standard- Weigh an amount of fluorescein corresponding to 1 g at the colouring matter content of sample. Dissolve in water and dilute to 100 ml. Make furher sequential dilutions as follows:

1 ml to 100 ml with water, 1 ml to 100 ml with water and 20 ml to 100 ml with solvent.

Chromatography solvent

N-Butanol:water:ammnia:ethanol = 100:44:1:22.5

TLC: Spot 25 ml of sample and standard solutions side by side on a cellulose plate. Develop for 16 h in the chromatography solvent. Allow the plate to dry. View under UV light and compare the fluorescence of the corresponding area on the chromatogram of the sample. The intensity of the latter shall not be greater than that of the former.

Organic compounds other than colouring matter

The method described as dye intermediates shall be followed :

2(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid-0.047 mg/L/cm at 348 nm (alkaline)

Tri-iodoresorcinol-0.079 mg/L/cm at 223 nm (acidic)

xi. Tartrazine

Purity

Weigh accurately about 250 mg of the dye sample and dissolve in 0.1N HCl in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 1mg per 100 ml. Find out the optical density of this diluted solution against 0.1N HCl solution as blank at 428 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 485}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

485 = Extinction coefficient for tartrazine in 0.1N HCl solution.

Subsidiary dye

The details of the method given above shall be followed.

Developing solvent No. 4

Height of ascent of solvent front= 12 cm.

xii. Sunset Yellow FCF

Purity

Weigh accurately about 250 mg of the dye sample and dissolve in 0.1N HCl in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 1mg per 100 ml. Find out the optical density of this diluted solution against 0.1N HCl solution as blank at 482 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 543}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

543 = Extinction coefficient for sunset yellow in 0.1N HCl solution.

Subsidiary dye

The details of the method given above shall be followed.

Developing solvent No. 4

Height of ascent of solvent front= 17 cm.

xiii. Indigo Carmine

Purity

Weigh accurately about 250 mg of the dye sample and dissolve with ammonium acetate solution in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 1 mg per 100 ml. Find out the optical density of this diluted solution against ammonium acetate solution as blank at 610 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 450}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

450 = Extinction coefficient for indigo carmine in ammonium acetate solution.

Subsidiary Dye

The details of the method given above shall be followed.

Developing solvent No. 3

Height of ascent of solvent front= 17 cm.

xiv. Brilliant Blue FCF

Purity

Weigh accurately about 100 mg of the dye sample and dissolve with ammonium acetate solution in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 0.2 mg per 100 ml. Find out the optical density of this diluted solution against ammonium acetate solution as blank at 630 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 1640}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

1640 = Extinction coefficient for brilliant blue FCF in ammonium acetate solution.

Subsidiary Dye

The details of the method given above shall be followed.

Developing solvent No. 4

Develop chromatogram for approx. 20 h.

xv. Fast Green FCF

Purity

Weigh accurately about 100 mg of the dye sample and dissolve with ammonium acetate solution in a 250 ml volumetric flask. Dilute this with the same solvent to make a final concentration of 0.2 mg per 100 ml. Find out the optical density of this diluted solution against ammonium acetate solution as blank at 625 nm in a glass cell with 10 mm path length. Simultaneously weigh accurately about 2 g of the dye sample and dry this in an air oven at 105±1°C for 2 h. Calculate the loss of mass on drying and from this data calculate the dry mass of the sample in the final solution taken for measurement of the optical density.

$$\text{Total dye, \% by mass} = \frac{OD \times 100}{M \times 1560}$$

Where,

OD = optical density found,

M = dry mass of sample in 100 ml of solution, and

1560 = Extinction coefficient for Fast green FCF in ammonium acetate solution.

Subsidiary Dye

The details of the method given above shall be followed.

Developing solvent No. 4

Height of ascent of solvent front= 12 cm.

Check Your Progress - 1

1. How will you check the purity of â-carotene and annatto colour?

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2. By which method the solid content of caramel colour in determined?

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3. How may spots are visible in day light and UV light respectively on a chromatogram of solution of saffron pigment in methanol.

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4. How Ammoni cal nitrogen % by mass is determined in caramel?

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5. What is the technique used to determine the subsidiary dyes in synthetic food colours?

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6. How the purity of tartrazine and sunset yellow is determined?

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15.3 ACIDULANTS

i. Lactic Acid

Lactic acid is used as acidulant in beverages like soft drinks, mineral water, carbonated fruit juice acid, pickles, jam, jellies, etc. It is also used in the manufacture of beer and wine, and dairy products like paneer, casein, chhana, processed cheese, unsalted butter and in processing of meat, egg whites and sauces of canned fish.

Purity

Weigh accurately a portion of the sample equivalent to 5 g of lactic acid, transfer to a 250 ml flask, add 50 ml of 1N NaOH, mix and boil for 20 min. Add phenolphthalein (Dissolve 0.2 g of phenolphthalein in 60 ml of 90 percent ethanol and add water to make 100 ml), and titrate the excess alkali in the hot solution with 1N sulphuric acid, and perform a blank determination. Each ml of 1N NaOH is equivalent to 90.08 mg of lactic acid.

ii. Phosphoric Acid

Purity

Weigh 1 g of phosphoric acid into a glass stoppered flask, dilute with about 100 ml of water, and add 0.5 ml of thymolphthalein solution (Dissolve 0.1 g of thymolphthalein in 100 ml of ethanol and filter, if necessary.) Titrate with 1N NaOH. Each ml of 1N NaOH is equivalent to 0.049 g of H_3PO_4.

iii. Citric Acid

Citric acid is used as an antioxidant, synergist, sequesterant, acidulant and flavouring agent. Its use has been permitted in the PFA rules.

Purity

Dissolve about 3 g of the accurately weighed sample in 40 ml of water. Add phenolphthalein (1 g in 100 ml alcohol) and titrate with 1N NaOH. Each ml of 1N NaOH is equivalent to 64.04 mg of citric acid.

15.4 SWEETENERS

A Natural Sweeteners

i. Lactose (Commercial)

The lactose is manufactured from the whey remaining after the manufacture of casein, cheese or chhana from milk. Lactose finds its use in dietary foods and pharmaceutical products.

Purity

Reagents

- Acid mercuric nitrate solution: Dissolve purified mercury in twice its weight of nitric acid and dilute with 5 volumes of water.
- Mercuric iodide solution: Dissolve 33.2 g of KI and 13.5 g mercuric chloride in 200 ml of glacial acetic acid and 640 ml of water.
- Phosphotungstic acid solution: 5% (m/v)

Procedure

Take two graduated flasks, one of 100 ml and the other of 200 ml capacity. Weigh accurately 65.8 g of the prepared sample into each flask. Add to each flask 20 ml of acid mercuric nitrate solution or 30 ml of mercuric iodide solution. To the 100 ml flask, add phopshotungstic acid solution and dilute to the mark with water. Shake both the flasks frequently during 15 min filter through dry filter paper and polarize.

Calculate percentage of lactose as follows:

Subtract reading of solution from 200 ml flask (using 400 mm tube) from reading of solution from 100 ml flask (using 200 mm tube). Multiply difference by 2. Subtract result from reading of solution from 100 ml flask; Divide result by 2.

Specific rotation

Accurately weigh 10 g of the sample and dissolve it in a beaker in about 60 ml of water. Cover with a watch glass and boil for 15 min. Allow to cool, add a few drops of dilute ammonium hydroxide of sp gravity 0.96 and make up to 100 ml in volumetric flask. Measure the optical rotation at 20°C.

$$\text{Specific rotation, degrees} = \frac{100a \times 100}{l \times c \times w}$$

Where,

a=observed optical rotation in degrees,

l= length in tube in decimeters,

c= weight, in g, of sample in 100 ml of water, and

w= lactose percent in the sample.

ii. Dextrose Monohydrate

Purity

Reagents

- Fehling's solution A: Aqueous solution of cupper sulphate (68.28 g $CuSO_4.5H_2O$ /L).
- Fehling's solution B: Aqueous solution containing 346 g of sodium potassium tartrate, and 100 g sodium hydroxide per litre.
- Standard dextrose solution: Dissolve 2.5 g of pure dextrose dried at 70°C under reduced pressure, in distilled water to make 1000 ml.
- Methylene blue indicator : 1% aqueous solution.

Procedure

Weigh sample to 500 ml volumetric flask to give 0.25 g dextrose per 100 ml. With pipette transfer 5 ml of each Fehling's solutions A & B into 250 ml conical flask. Add pumice stone and enough water suitable for titration. Put the conical flask on the heating device. Right from the start of heating add sample or standard dextrose solution amounting within 0.5 ml of the anticipated end point. Adjust the heating in such a way as to make the solution boil within 2.75±0.25 min and then make no more adjustment till the end of the test. After 2 min of boiling, add 2 drops of methylene blue solution and complete the titration drop by drop. Carry out the operation till the blue colour vanishes. Titration should be completed within 1.5-2.0 min after the addition of the indicator.

$$\text{Dextrose, \% by mass} = \frac{125V}{VI \times M}$$

Where,
V=volume, in ml, of standard dextrose solution,
V1= volume, in ml, of the test solution used, and
W= mass, in g, of the test portion.

iii. Refined Sugar

Reducing sugar

The complex formed between Cu^{++} ions and potassium tartrate is reduced by reducing sugars to Cu^{+} ions which is precipitated as Cu_2O. The precipitated Cu_2O is the determined by iodometric titration. The Cu_2O is oxidized by an excess is back titrated with sodium thiosulphate. The reaction between the reducing sugars and Cu^{++} complex is not stoichiometric. The amount of Cu_2O formed depends upon the prescribed conditions which therefore strictly followed.

Reagents

- Modified Ofner solution : Weigh out 7 g of copper sulphate pentahydrate, 10 g sodium carbonate, 10 g 300 g potassium sodium tartrate and 50 g disodium hydrogen phosphate in a 1000 ml flask. Dissolve in approximately 900 ml water.

Heat the solution for 2 h in a boiling water bath. Cool down to room temperature and fill up to the mark. Add approximately 10 g activated carbon and stir for 5-10 min. Filter the solution.

- Potassium iodate solution (0.01667M)
- Starch solution (1%)
- Sodium thiosulphate solution (0.0333M)
- Iodine solution (0.01667M)

Procedure

Weigh 40 g sugar sample and make up with water to 200 ml (£ 25 mg invert sugar/ 50 ml).

Hot value : Mix 50 ml of the prepared solution with 50 ml Ofner solution. Add some pumice pieces to the mixture. Bring the mixture to the boil within 4-5 min. Boil for exactly 5 min. Cool the mixture down in a water bath with cold running water. After approximately 10 min the mixture should have reached room temperature. Add iodine solution until the colour of the mixture turns a typical iodine colour. This procedure dissolves the formed cuprous oxide with an excess of iodine. The surplus iodine should be so high that between 10-15 ml of sodium thiosulphate are consumed on back titration. Add 15 ml of 1M HCl by pouring it down the inner side of the flask so that the residual droplets are washed down into the solution. Cover the flask with a watch glass and move it gently for 2 min until the precipitate of cuprous oxide is completely dissolved. Titrate the sample with 0.0333M sodium thiosulphate. Add 1 ml of starch solution immediately before the end point is reached.

Cold value : Mix 50 ml of the prepared sample with 50 ml of the Ofner solution. Leave the mixture at room temperature for 10 min. Repeat the procedure as in hot value.

Blank value : Mix 50 ml of water with 50 ml of Ofner solution. Repeat the procedure as in hot value.

$$\text{Reducing sugar, mg/kg} = \frac{(A - B - C - D) \times 1000}{S}$$

Where,

A= calculated hot value; B= calculated cold value;

C= calculated blank value; D= sucrose correction, and

S= amount of sample in 50 ml of prepared solution.

Calculated cold/hot/blank value = (Vi×Fi-Vth×Fth)

Where,

Vi & Vth = volume of iodine solution and thiosulphate solution used, respectively.

Fi & Fth = correction factors for iodine solution and thiosulphate solution, respectively.

iv. Sucrose

Weigh accurately about 5 g of the sample in a beaker, add about 50 ml of water and warm the mixture in a water bath at 50-60°C for about 5 min to dissolve the sucrose content of the sample. Cool and filter through a Whatman filter paper No. 40. Collect the filtrate carefully in a 100 ml volumetric flask. Wash the beaker and the insoluble residue of starch in the filter paper carefully with water. Make up the volume of the filtrate to 100 ml.

Inversion : Take 10 ml of this solution in a conical flask and add 1.5 ml of conc HCl and about 10 ml of water. Heat the flask at 60-70°C for 10 min in a water bath. Cool immediately and transfer quantitatively the inverted solution to a volumetric flask and make up the volume to 100 ml.

Reducing Sugar : Pour the prepared solution into a 50 ml burette. Pipette 25 ml of Fehling's solution into 250 ml conical flask and run in from the burette 15 ml of the prepared solution. Without further dilution, heat the contents of the flask over wire gauze, and boil. When it judged that nearly all the copper is reduced, add 1 ml of methylene blue indicator solution. Continue boiling the contents of the flask for 1-2 min from the commencement of ebullition and then add the prepared solution n small quantities (1 ml or less at a time), allowing the liquid to boil for about 10 sec between successive additions, till the blue colour of the indicator just disappears. Note the reading of the burette and calculate the volume of the prepared solution used up in titration.

$$\text{Sucrose, \% by mass} = \frac{0.95(Q - WR)}{W}$$

Where,

Q = value in table corresponding to the titre,

R= percent of reducing sugar by mass, and

W= mass, in g, of the material taken for the test.

v. Honey

Specific Gravity

Clean and thoroughly dry the specific gravity bottle and weigh. Fill it up to the mark with freshly boiled and cooled distilled water which has been maintained at 27±1°C and weigh. Remove the water, dry the bottle again and fill it with the honey sample maintained at the same temperature. Weigh the bottle again.

$$\text{Specific gravity at 27°C} = \frac{\text{Weight of honey in bottle}}{\text{Weight of water in bottle}}$$

Moisture

Determine the refractometer reading of honey at 20°C and calculate the percentage of moisture from the values given in table.

Fructose-Glucose Ratio

Pipette 50 ml of honey solution in a 250 ml flask. Add 40 ml of iodine solution and 25 ml of sodium hydroxide solution. Stopper the flask and keep in dark for 20 min. Acidify with 5 ml of sulphuric acid and titrate quickly the excess of iodine against standard sodium thiosulphate solution. Conduct a blank using 50 ml of water instead of honey solution.

$$\text{Approx. glucose, \% by mass (G)} = \frac{(B - S) \times 0.004502 \times 100}{W}$$

Where,

B = volume of sodium thiosulphate solution required for the blank,

S= volume of sodium thiosulphate solution required for the sample, and

W= mass, in g, of honey taken for test.

$$\text{Approx. fructose, \% by mass (F)} = \frac{(\text{Approx. reducing sugar, \% - G})}{0.925}$$

True glucose, % by mass (D) = G-0.012F

$$\text{True fructose, \% by mass} = \frac{(\text{Approx. reducing sugar, \% - D})}{0.925}$$

B. Artificial Sweetners

i. Aspartame

Aspartame is a non-nutritive sweetener, sugar substitute and flavour enhancer. It is 100-200 times sweeter than sucrose and has an acceptable dietary intake of 0-40 mg/kg body weight. It has been permitted for use under the PFA rules.

Purity

Weigh accurately about 150 mg of sample, previously dried at 105°C for 4 h. Dissolve in 35 ml of dimethylformamide. Add 5 drops of thymol blue and titrate with a micro burette to a dark blue end point with 0.1M lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1M lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

Note : Sodium methoxide may be used for the titration.

5-Benzyl-3,6-Dioxo-2-Piperazine Acetic acid

Gas Chromatograph

GC equipped with FID containing a 1.83 m×4 mm I.D. glass column packed with 3 percent OV-1 on 80/100 mesh Supelcoport. Condition the column overnight at 250°C before readjustment and equilibration to the operating condition.

Operating Conditions

The operating parameters may vary depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

(a) Column temperature : 200°C

(b) Injector temperature : 200°C

(c) Detector temperature : 275°C

(d) Carrier gas-N2 flow rate : 75 ml/min

(e) H2 and air flow to burner : Optimized to give maximum sensitivity.

(f) Recorder : 1 mv full scale

Reagents

1. Silation reagent: Just before use, dilute 3 parts by volume of N,O-bis-trimethylsilyl) acetamide with 2 parts of dimethylformamide.

2. Standard solution: Transfer 25 mg of 5-Benzyl-3,6-dioxo-2-piperazine acetic acid into a 50 ml volumetric flask, dissolve in methanol dilute to volume with methanol and mix. Pipette 10 ml of this solution into a 100 ml volumetric flask dilute volume with methanol and mix. Pipette 3 ml of second solution into a 2 dram vial with Teflon lined cap and evaporate to dryness on a steam bath. Add 1 ml of silation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80°C for 30 min. Remove the vial from the oven, shake for 15 sec and cool to room temperature.

3. Sample preparation: Transfer about 10 mg of aspartame sample accurately weighed into a 2 dram vial with Teflon lined cap, add 1 ml of the silation reagent, cap tightly, shake and heat in an oven at 80°C for 30 min. remove the vial from the oven, shake for 15 sec and cool to room temperature.

Procedure

Inject a 3 ml portion of standard preparation into the GC obtain the chromatogram, measure the height of peak produced by 5-benzyl-3,6-dioxo-2-piperazxine acetic acid. Under the stated conditions, the elution time is about 7-9 min. Similarly inject a 3 ml portion of sample preparation, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3,6-dioxo-2-piperazxine acetic acid contained in the sample.

ii. Sodium Saccharin

Sodium saccharin (sodium salt of 2,3-dihydro-oxobenzisulfonazole or 1,2-benzisothiazolin-3-one-1,1-dioxide sodium salt dihydrate is a non-nutritive sweetener. It is 500 times sweeter than sucrose. It is used in specifically notified food products under PFA rules as artificial sweetener within the prescribed limits.

Purity

Dissolve about 0.3 g of previously dried sample, accurately weighed in 20 ml glacial acetic acid. Add 2 drops of crystal violet-glacial acetic acid indicator and titrate with 0.1N perchloric acid. End point shall be when violet colour of solution

change to green via blue. Perform a blank determination, make any necessary correction, Each ml of 0.1N perchloric acid is equivalent to 20.52 mg of sodium saccharin.

Toluenesulfonamides

Reagents

- Solution A : 4 volumes of methanol plus 1 volume of acetone plus 0.5 percent (m/v) of the sample.
- Solution B: 4 volumes of methanol plus 1 volume of acetone plus 0.005% (m/v) of 4-sulphamoylbenzoic acid.
- Solution C : 4 volumes of methanol plus 1 volume of acetone plus 0.005% (m/v) of toluene-2-sulfonamide.

Procedure

Prepare silica gel G plate (0.25 mm thick) and dry plates at 105-110°C for 1 h. Pour into the developing chamber sufficient quantity of mobile phase (100 volumes of chloroform+ 50 volumes of methanol+11.5 volume of strong ammonia solution) to form a layer about 15 mm deep. Close the tank for 1 h at 20-27°C. Using micropipette apply separately to the chromatoplates 2 ml each of solutions A, B and C.

Dry the spots and place the chromatoplates in the developing chamber at 20-27°C until the mobile phase has ascended to the 15 cm line. Remove the plates and dry them in current of warm air. Then heat at 105°C for 5 min. Spray the hot plates with the sodium hypochlorite solution (0.5% m/v). Dry in a current of cold air until sprayed area of the plate below the line of application give at most a faint blue colour with a drop of a mixture, prepared by dissolving 0.5% KI in starch mucilage containing 1% m/v of glacial acetic acid. Spray the plates with the same mixture. The spots in the chromatograms obtained with solution B and C should be more intense than any corresponding spots in the chromatogram obtained with solution A.

iii. Sorbitol Powder

Sorbitol powder (food grade) used as a food additive is permitted for use in certain foods under PFA rules.

Purity

Weigh accurately about 50 mg of sample and mix with 40 ml of sodium periodate solution and allow to stand for 1 h. Then add 1 g of potassium hydrogen carbonate, 50 ml sodium arsenite solution and about 2 g of KI. Titrate against iodine solution using starch as indicator. Carry out a blank. The difference between the two titrations corresponds to the volume of sodium periodate taken up by the sorbitol powder. Each ml of 0.1N sodium periodate corresponds to 0.0018218 g of sorbitol.

Check Your Progress – 2

1. How the purity of lactic acid, phosphoric acid and citric acid is determined?

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2. Generally at what temperature the optical rotation of a sugar solution is determined?

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3. Write the formula used to calculate the concentration of lactose in solution from its optical rotation.

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4. Which are the reagents used to determine the purity of dextrose monohydrate? Name the indicator used for the titration?

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5. How sucrose in solution is converted into invert sugars?

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6. What is the importance of fructose-glucose ratio in honey?

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15.5 ANTIOXIDANTS

i. Ascorbic Acid

Purity

Dissolve about 400 mg of accurately weighed ascorbic acid, previously dried in a vacuum desiccator over sulphuric acid for 3 h, in a mixture of 100 ml of water and 25 ml of dilute sulphuric acid. Titrate the solution at once with 0.1N iodine, adding a few drops of starch indicator (triturate 0.5 g of starch with 5 ml of water and add this, stirring constantly, to sufficient water to make up about 100 ml. Boil for a few minutes, cool and filter. Starch solution shall be freshly prepared.) as the end point is neared. Each ml of 0.1N iodine is equivalent to 0.008806 g of $C_6H_8O_6$.

ii. Butylated Hydroxyanisole

Butylated hydrioxyanisole (BHA), food grade is used as an antioxidant. BHA is permitted for use in foods under the PFA rules.

Purity

Prepare a solution of pure BHA in 80% ethanol containing 5.0 mg/ml. Place suitable aliquots (1-12 ml) of BHA solution into small glass stoppered bottles to give a range of 5-60 mg/aliquot. Add enough 80 % ethanol to each bottle to give a total of 12 ml. Then add 2 ml of aqueous borax and 2 ml of 2,6-dichloroquinonechlorimide. Age the samples and the blank for 15 min. Using the blank as a reference standard; determine the optical density at 610 nm on a spectrophotometer. Proceed as above using a sample solution in 80% ethanol. From the optical density, find out the purity of BHA using the standard curve.

Phenolic Impurities

Phenolic impurities are determined by the method using silica gel G plates.

- Solution 1 : Dissolve 0.25 g of BHA in 10 ml of ether.
- Solution 2 : Dilute 1 ml of solution 1-10 ml with ether and then dilute 1 ml of this solution to 20 ml with ether. Use the final dilution as solution 2.

Spot 2 ml of each of solution 1 and solution 2 on separate TLC plates and properly identify them. Place them in developing chamber containing chloroform as solvent and allow the solvent to ascend to a point of 15 cm above the sample spots. Develop the chromatograms by spraying a mixture containing 100 ml of 10.5 % ferric ferrocyanide solution and 25 ml of 5 % ferric chloride solution. Any blue violet spots appearing on chromatogram 1 (other than major spot and the spot of Rf 0.35) are not more intense than the major spot appearing on chromatogram 2.

Specific Absorption

Prepare 1% solution of BHA in ethanol and find out its specific absorption in a suitable spectrophotometer using 1 cm cell at 290 nm and 228 nm.

iii. Propyl Gallate

Use of propyl gallate, food grade is permitted under the PFA rules as an antioxidant in edible oils and fats except ghee and butter.

Purity

Reagents

- Bismuth nitrate (Dissolve 5 g of bismuth nitrate in 25 ml of water and 25 ml of glacial acetic acid and dilute to 250 ml.)
- Acetic acid (0.1N)
- Nitric acid (0.05N)
- Acetone

Procedure

Dry a suitable quantity of sample in an oven at 90°C for 6 h, cool it in a desiccator. Weigh 100 mg of propyl gallate into a 250 ml beaker, add 15 ml of acetone, 10 ml of water and bring to the boiling point. Add 10 ml of bismuth nitrate and bring again to the boiling point. Cool to room temperature. Filter through a tarred sintered glass crucible. Wash twice with acetic acid and twice with water. Wash six times with nitric acid at 80°C pressing the precipitate down well between each wash. Wash twice with water. Dry at 110°C to constant mass.

$$\text{Propyl gallate, \% by mass} = \frac{\text{Mass of precipitate} \times 0.4863 \times 100}{\text{Mass of sample}}$$

iv. Octyl Gallate

Purity

Reagents

- Bismuth nitrate (Dissolve 5 g of bismuth nitrate in 25 ml of water and 25 ml of glacial acetic acid and dilute to 250 ml.)
- Acetic acid (0.1N)
- Nitric acid (0.05N)
- Acetone

Procedure

Dry a suitable quantity of sample in an oven at 90°C for 6 h, cool it in a desiccator. Weigh 100 mg of octyl gallate into a 250 ml beaker, add 15 ml of acetone, 10 ml of water and bring to the boiling point. Add 10 ml of bismuth nitrate and bring again to the boiling point. Cool to room temperature. Filter through a tarred sintered glass crucible. Wash twice with acetic acid and twice with water. Wash six times with nitric acid at 80°C pressing the precipitate down well between each wash. Wash twice with water. Dry at 110°C to constant mass.

$$\text{Octyl gallate, \% by mass} = \frac{\text{Mass of precipitate} \times 0.5574 \times 100}{\text{Mass of sample}}$$

Chlorinated Organic Compounds

Dissolve 1 g of the sample in 10 ml of 0.1N NaOH. Acidify with nitric acid solution and filter off the precipitate. Mix the precipitate with 2 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid and filter. Mix the solution with 0.5 ml of 0.1 N silver nitrate. The turbidity should not be more than that obtained in 20 ml of dilute nitric acid by addition of 0.5 ml of 0.1N silver nitrate and 0.3 ml of 0.01N HCl.

Free Acid (as gallic acid)

To a mixture of 50 ml carbon dioxide free water and 50 ml of acetone, add 5 drops of bromocresol green and titrate with 0.005N HCl to match a buffer containing the same amount of indicator. Dissolve 0.4 g of sample in 50 ml of acetone and add 50 ml of water, 5 drops of bromocresol green and the amount of 0.005N HCl found in the preliminary test to bring the solvent to pH 5. Titrate the solution back to pH 5 with 0.05N NaOH, matching against the buffer. Each ml of 0.05N NaOH is equivalent to 8.506 mg of gallic acid.

Specific Absorption

Prepare 1% solution of the sample in ethanol and find out its specific absorption in a suitable spectrophotometer using 1 cm cell at 275 nm.

v. Dodecyl Gallate

Purity

Reagents

- Bismuth nitrate (Dissolve 5 g of bismuth nitrate in 25 ml of water and 25 ml of glacial acetic acid and dilute to 250 ml.)
- Acetic acid (0.1N)
- Nitric acid (0.05N)
- Acetone

Procedure

Dry a suitable quantity of sample in an oven at 90°C for 6 h, cool it in a desiccator. Weigh 100 mg of dodecyl gallate into a 250 ml beaker, add 15 ml of acetone, 10 ml of water and bring to the boiling point. Add 10 ml of bismuth nitrate and bring again to the boiling point. Cool to room temperature. Filter through a tarred sintered glass crucible. Wash twice with acetic acid and twice with water. Wash six times with nitric acid at 80°C pressing the precipitate down well between each wash. Wash twice with water. Dry at 110°C to constant mass.

$$\text{Dodecyl gallate, \% by mass} = \frac{\text{Mass of precipitate} \times 0.6017 \times 100}{\text{Mass of sample}}$$

vi. Ascorbyl Palmitate

Use of ascorbyl palmitate, food grade is used as an antioxidant permitted under the PFA rules in edible oils and fats except ghee and butter.

Purity

Dissolve about 300 mg accurately weighed material in 50 ml of alcohol in a 250 ml Erlenmeyer flask. Add 30 ml of water and immediately titrate with 0.1N iodine solution to a yellow colour which persists for minimum 30 sec. Each ml of 0.1N iodine solution consumed is equivalent to 20.73 mg of ascorbyl palmitate.

Check Your Progress – 3

1. What technique is used to determine the phonetic impurities is BHA?

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2. What method is used to determine the purity of propyl and octyle gallates?

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15.6 EMULSIFYING AND STABILIZING AGENTS

i. Lecithin

Lecithin, food grade widely used as an antioxidant and emulsifier, is permitted under the Prevention of Food Adulteration rules (1955) and the Fruit Product Order (1955).

Purity

Dissolve 5 g of phophatides from previous acetone insoluble matter in 10 ml of petroleum ether and add 25 ml of acetone to the solution. The precipitate portions are transferred to 4 centrifuge tubes with additional amount of acetone, stir again, chill for 15 min in an ice bath, stir again and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refilled with acetone and repeat the centrifuge. 5 g of the purified phosphatides are required to saturate about 16 L of acetone.

Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°C to form a saturated solution, and maintain the mixture at this temperature for 2 h, shaking it vigorously at 15 min intervals.

Transfer 2 g of sample accurately weighed into a 40 ml centrifuge tube, previously tarred with a glass stirring rod. Add 15 ml of phosophatide-acetone solution from

a burette. Warm the mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Chill for 5 min, add about one-half of the required volume of phosphatide-acetone solution and stir for 15 min. Centrifuge the content at 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the stirring rod. Repeat the process. Mix the residue again, dry the centrifuge tube and its contents at 105°C for 45 min in oven, cool and weigh.

ii. Sodium Alginate

Sodium alginate widely used as stabilizing and thickening agent, is permitted under PFA rules

Purity

Apparatus : Soda lime column (A), a mercury valve (B) connected through a side tube (C) with a reaction flask (D) by means of a rubber connection, the reaction flask (D) is a 100 ml round bottom long necked boiling flask with 24/40 ground joint attached. The oil bath (E) is maintained at 145°C by means of a thermo-regulator and an immersion heater.

The reaction flask is provided with a 20 cm reflux condenser (F) terminating in a trap (G) containing 25 g of 850 m zinc or tic, which is connected with an absorption flask (H) a 250 ml Erlenmeyer flask equipped with a 24/40 ground joint and a side tube attached a little below the ground joint. Flask (H) is provided with an absorption tower (J) the lower part of which consists of an 18 mm tube fitted with a medium porosity fritted Pyrex, sealed to the inner part of the lower end of a 24/40 ground joint and terminating 1-2 mm above the bottom of the absorption flask when the joint is in place.

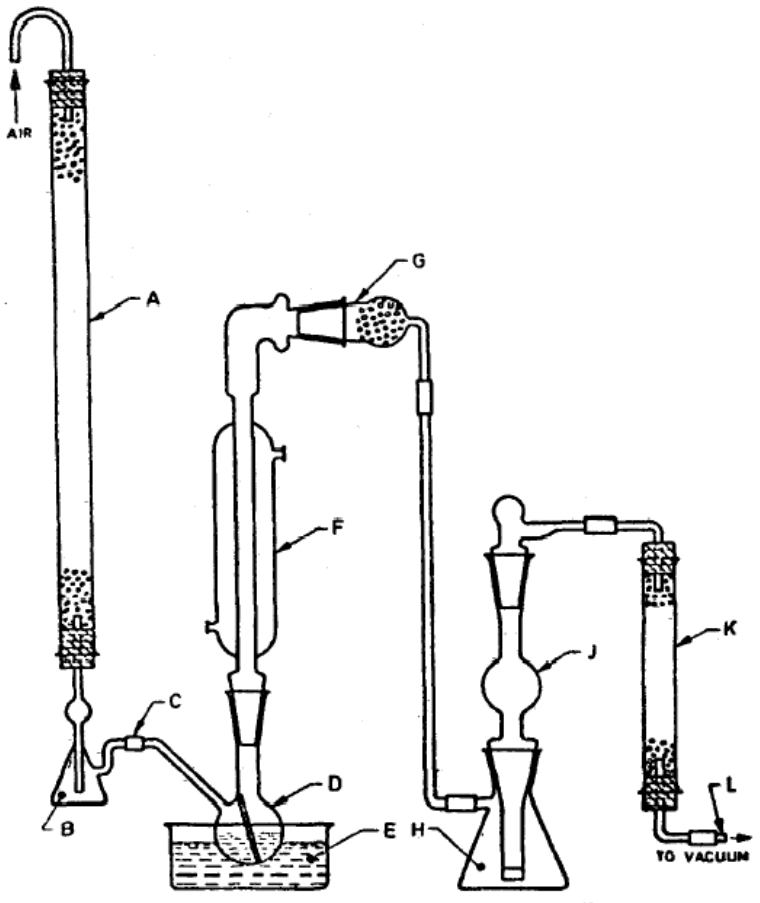

Fig 15.2 Appratus for Determination of Purity

A trap consisting of a bulb of 100 ml capacity, is blown above the ground portion of the joint and the outer portion of 1 24/40 ground joint is sealed on above this bulb. The absorption tower, from the botto0m of the disc to the top of ground joint is 30 cm in length. The top of the tower is fitted with a hollow ground stopper with a short side-tube attached. The tower assembly may be attached to a soda lime tower connected with a water pump by means of capillary tube regulator (L) which serves to seep 1700 to 2000 ml of carbon dioxide free air per hour through the apparatus during the heating period (Figure 1)

Procedure

Transfer about 250 mg of sample previously dried at 105°C for 4 h and accurately weighed to the reaction flask, add 30 ml of 19% HCL, insert a small boiling tube and connect it to the reflux condenser (F) using syrupy phosphoric acid as lubricant.

Draw a carbon dioxide free air for about 10 min and then discontinue it. Disconnect the absorption tower (J) rapidly transfer from a pipette 25 ml of 0.25N NaOH into the absorption flask, add 5 drops of butanol and again attach it to the absorption tower. Raise the oil bath, previously heated to 145°C until the oil level is several mm above the liquid level in the reaction flask. After the initial rapid evolution of carbon dioxide has subsided, resume the passage of air through the apparatus and continue the heating at 145°C for 2 h, then disconnect the absorption flask and rest of the assembly. Remove the absorption tower, washing any adhering NaOH solution into the flask with several small portions of water. To the flask add 10 ml of barium chloride solution (1:10), stopper the flask, shake gently for about 2 min, add phenolphthalein solution and titrate with 0.1N HCl. Perform a blank determination make necessary correction.

Each ml of 0.25N NaOH is equivalent to 27.75 mg of sodium alginate (equivalent wt 222.0)

iii. Sodium Carboxymethyl Cellulose (CMC)

Sodium carboxymethyl cellulose, food grade widely used as thickening agent, emulsifier and stabilizer, is permitted under the Prevention of Food Adulteration rules (1955) for certain foods.

Degree of Substitution

Weigh 500 mg of sodium CMC in a beaker. Add 80 ml of glacial acetic acid. Heat the mixture on a boiling water bath for 2 h and cool to room temperature. Set the pH meter on mV mode and deep the electrode in the solution. Take a definite volume of perchloric acid in a graduated burette and add to the solution in large increments until the change on mV becomes noticeable. Then add at the rate of 0.1 ml and note the mV reading each time. Continue addition as above until the variation in the readings passes through a maximum. Plot the amount of perchloric acid against the mV readings and determine the quantity of titrant corresponding to half-way up the steepest gradient.

$$\text{Degree of substitution} = \frac{16.2(A/M)}{1.0-[8.0(A/M)]}$$

Where,

A = volume in ml of 0.1N perchloric acid required, and

M = mass in mg of the sample taken for the test.

Sodium Chloride

Weigh accurately about 5 g of the sample into a 250 ml beaker. Add 50 ml of water and 5 ml of hydrogen peroxide and heat on a steam bath for 20 min, stirring occasionally to ensure complete dissolution. Cool and add 100 ml of water and 10 ml of nitric acid. Titrate with the silver nitrate to a potentiometric end point, using silver and mercurous sulphate- potassium sulphate electrodes with continuous stirring.

$$\text{Sodium chloride (as NaCl), \% by mass} = \frac{584.5 \times N \times V\,(100 - A)}{M}$$

Where,

V= volume in ml of silver nitrate used in the titration with the material, corrected to blank;

N = normality of standard silver nitrate solution;

A= percent loss on drying, and

M = mass in g of sample taken for the test.

Free Glycolate

Weigh 0.5 g of the sample and transfer to a 100 ml beaker. Moisten the sample thoroughly with 5 ml of glacial acetic acid, followed by 5 ml of water, and stir with a glass rod until the solution is complete; usually about 15 min are required. Slowly add 50 ml of acetone while stirring and the 1 g of NaCl. Continue the stirring for several minutes to ensure complete precipitation of the CMC. Filter through a soft, open-texture paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100 ml volumetric flask. Use 30 ml of acetone to facilitate the transfer of the solids and to wash the filter cake. Make up to volume with acetone and mix.

Prepare a blank solution containing 5 ml of water, 5 ml of glacial acetic acid and acetone in another 100 ml volumetric flask. Pipette 2 ml of the sample solution and 2 ml of blank solution into two 25 ml volumetric flasks. Remove the acetone by heating the uncovered flasks upright in a boiling water bath for exactly 20 min. Cool to room temperature and add 5 ml of naphthalenediol, mix thoroughly, then add 15 ml more of the naphthalenediol and mix. Cover the mouth of the flask with a small piece of aluminium foil and heat upright in the boiling water bath for 20 min. Cool to room temperature and make up to volume with conc sulphuric acid. Measure the absorbance of sample solution against blank solution at 540 nm using 1 cm cell. Read the corresponding mg of glycolic acid from the calibration curve obtained as follows:

Introduce 0, 1, 2, 3 and 4 ml aliquots of standard glycolic acid solution (1mg/ml) prepared by weighing accurately 0.1 g of glycolic acid, previously dried in a vacuum desiccator for at least 16 h and then dissolving in 100 ml of water; do not keep the solution longer than 30 days. Add water to each flask to a volume

of 5 ml, then add 5 ml glacial acetic acid and make up with acetone to mark and mix. Pipette 2 ml of each solution into a series of 25 ml volumetric flasks and proceed in the same manner as described for the test solution.

$$\text{Sodium glycolate, \% by mass} = \frac{A \times 0.129}{B}$$

Where,

A= mg of glycolic acid read from the calibration curve, and

M = mass in g of dried sample taken for the test.

iv. Guar Gum

Guar gum is obtained from ground endosperms of *Cyamopsis tetragonolobus*. It consists chiefly of a high molecular weight hydrocolloidal polysaccharide, galactomannan. Use of guar gum as food additive is permitted under the Prevention of Food Adulteration rules (1955).

Identification of Sugars

Boil a mixture of 100 mg of sample and 20 ml of 10% sulphuric acid for 3 h. Allow to cool and add excess barium carbonate (about 10 mg). Mix with a magnetic stirrer until the solution is of pH 7, and filter. Evaporate the filtrate in a rotary evaporator at 30-50°C in vacuum until a crystalline residue is obtained. Dissolve it in 10 ml of 40% methanol.

Prepare a thin layer chromatoplates with a mixture of 15 g cellulose powder and 90 ml water and dry them for 10 min at 100°C.

Place 1-10 ml spots of hydrolysate on the starting line of two chromatoplates and spots containing 1-10 mg of the sugars which could be present in the hydrolysate. Use two solvents (a) a mixture of formic acid, methyl ethyl; ketone, tertiary butanl and water (15:30:40:15, v/v) and (b) a mixture of isoprapanol, pyridine, acetic acid and water (40:40:5:20, v/v) to develop the plates.

After development, spray with a solution of 1.23 g anisidine and 1.66 g phthalic acid in 100 ml ethanol and heat the plates at 100°C for 10 min. A greenish-yellow colour is produced with hexoses, a red colour with pentoses and a brown colour with uronic acids. Compare sample spots with those for the solution of galactose and mannose.

Galactomannans

The difference between the sum of the percentages of acid insoluble matter (1% solution in 0.1% sulphuric acid), total ash at 550°C, loss on drying at 105°C for 5 h and protein (N×5.7) and 100 represents the percent of galactomannans.

v. Gum Ghatti

Gum ghatti is a complex polysaccharide composed of l-arabinose, d-galactose, d-mannose and d-glucoronic acid residues. It is used as an emulsifier, stabilizer and thickener in foods and pharmaceuticals. It is a water-soluble gum exuded by the tree *Anoqeissus latifolia* of the family combretaceae.

Viscosity

Weigh accurately 5 g of sample in a 250 ml beaker. Fix a stirrer and thermometer in it. Add 10 ml iso-propyl alcohol and mix it thoroughly so as to form a slurry. Add 80 ml of boiling water quickly while stirring the solution rapidly. If there are any lumps in the solution, discard and prepare the fresh solution until a clear solution is obtained. Cool and stir the solution till the temperature drops to 80°C. Place the beaker in a water bath maintained at about 85°C and stir frequently for 10 min. Remove the beaker and place it in a water-bath maintained at 27±1°C. Stir the solution and add water so that the final weight of the beaker is 100 g more than the tare weight of the beaker. Adjust the temperature of the solution to 27±1°C and measure its viscosity with Brookefield viscometer at 2o rpm using spindle No. 4.

vi. Tragacanth Gum

Use of tragacanth gum as thickening agent and stabilizer has been permitted in the PFA rules for certain foods. It is dried gummy exudation obtained from *Astragalus strobiliferus.*

Chromatographic identifications of sugars in the gum hydrolysate

Boil a mixture of 100 mg of sample and 20 ml of 10% sulphuric acid for 3 h. Allow to cool and add excess barium carbonate (about 10 mg). Mix with a magnetic stirrer until the solution is of pH 7, and filter. Evaporate the filtrate in a rotary evaporator at 30-50°C in vacuum until a crystalline residue is obtained. Dissolve it in 10 ml of 40% methanol.

Prepare a thin layer chromatoplates with a mixture of 15 g cellulose powder and 90 ml water and dry them for 10 min at 100°C.

Place 1-10 ml spots of hydrolysate on the starting line of two chromatoplates and spots containing 1-10 mg of the sugars which could be present in the hydrolysate. Use two solvents (a) a mixture of formic acid, methyl ethyl; ketone, tertiary butanl and water (15:30:40:15, v/v) and (b) a mixture of isoprapanol, pyridine, acetic acid and water (40:40:5:20, v/v) to develop the plates.

After development, spray with a solution of 1.23 g anisidine and 1.66 g phthalic acid in 100 ml ethanol and heat the plates at 100°C for 10 min. A greenish-yellow colour is produced with hexoses, a red colour with pentoses and a brown colour with uronic acids.

Viscosity

Transfer a 4 g sample, finely powder into the container of a stirring apparatus equipped with blades capable of revolving at 1000 rpm. Add 10 ml of alcohol to the sample, so as to wet the gum uniformly, and then add 390 ml of water, avoiding the promotion of lumps. Immediately stir the mixture for 7 min, pour the resulting dispersion into a 500 ml bottle, insert a stopper, and allow to stand for about 24 h in a water bath at 25°C. Determine the apparent viscosity at a temperature with a Brookefield viscometer using spindle No. 2 at 3 rpm and a factor 10.

Test for Karaya Gum

Boil 1 g of the material with 20 ml of water until mucilage is formed, add 5 ml of HCl and again boil the mixture for 5 min. No permanent pink or red colour shall develop.

vii. Gelatin

Gelatin is a protein produced by partial hydrolysis of collagen derived from skin, tendons, ligaments and bones of animals. Gelatin is widely used in the food processing industry as a stabilizer, gelling agent, emulsifying agent and a crystallization inhibitor. It is permitted as a food additive under the PFA rules (1955).

Gel strength

Weigh accurately about 1 g and place with 99 of water in a 200 ml flask. Allow to stand for 15 min; then place the flask in a water bath at 60°C and swirl occasionally until solution is complete. Transfer 10 ml of the solution to a test tube having an internal I.D. of 12 mm and place the tube in ice bath, making certain that the top of the solution is below the level of the ice and water. Place the bath containing the tube in a refrigerator, and maintain it at about 0°C for 6 h. When the tube is removed from the bath and inverted, no movement of the gel shall be observed.

viii. Sodium Citrate

Sodium citrate, food grade widely used as emulsifying and stabilizing agent, is permitted under the Prevention of Food Adulteration rules (1955) for certain foods.

Purity

Weigh accurately about 2 g of the sample. Heat until carbonized. Cool. Boil the residue with 50 ml of water and 50 ml of the sulphuric acid. Filter and wash the filtrate with water. Titrate the excess of acid in the filtrate and washings with the NaOH using solution of methyl orange as indicator. Each ml of 0.5N sulphuric acid is equivalent to 0.04902 g of $C_6H5O_7Na_3.2H_2O$.

ix. Dicalcium Phosphate

Dicalcium phosphate has been permitted as stabilizer, dough conditioner and as nutrient supplement in certain foods.

Purity

Weigh accurately about 400 mg of the sample, dissolve in 150 ml of water, and add 15 ml of NaOH and 300 mg hydroxynaphthol blue indicator. Titrate with disodium ethylenediamine tetraacetate until the solution is clear. Each ml of 0.05M disodium EDTA is equivalent to mg of $CaHPO_4$.

x. Edible Common Salt (Dairy Salt)

Purity

Accurately weigh about 20 g of the dried sample, dissolve it in 200 ml of water

in a beaker and heat to boiling and cool. Filter the solution through a weighed Gooch or sintered glass crucible (G No. 4) and wash the residue till it is free from soluble salts. Collect the filtrate and washings in a 1 litre graduate flask and dilute to mark. Transfer 10 ml of the solution into a conical flask and add 1 ml of potassium chromate indicator solution. Titrate against standard silver nitrate solution till the reddish brown tinge persists after brisk shaking. Carry out a blank determination.

$$\text{Sodium chloride (as NaCl), \% by mass} = \frac{584.5 \times N \times V}{M}$$

Where,

V= volume in ml of silver nitrate used in the titration with the material, corrected to blank;

N = normality of standard silver nitrate solution; and

M = mass in g of the dried sample in 1000 ml of the solution taken for the test.

xi. Glyceryl Monostearate

Use of glyceryl monostearate is permitted as emulsifier for certain food products in the Prevention of Food Adulteration rules (1955). It shall preferably be made from edible vegetable oils.

Monoglyceride

The method is based upon their quantitative oxidation with periodic acid. Quantitative oxidation is obtained only if excess of periodic acid is used. The monoglyceride or glycerine is determined by the periodic acid consumed.

Reagents

- Periodic acid stock solution (Dissolve 12 g of periodic acid in water and dilute to 100 ml. Store in an amber colour bottle)
- Reagent solution (Dilute 5 ml of stock solution to 100 ml with 95% acetic acid)
- Starch indicator (1%)
- Chloroform
- Acetic acid (Dilute redistilled glacial acetic acid to 95%)
- Potassium iodide solution (15% in water)
- Standard sodium thiosulphate solution (0.1N)

Procedure

Weigh 0.7-0.8 g sample into 100 ml glass stoppered volumetric flask accurately and rapidly. Add 50 ml chloroform. Gently swirl to dissolve the sample. If the sample is not soluble (due to the presence of soap) add 5 drops of acetic acid and shake. Add 25 ml distilled water and stopper the flask. Shake vigorously for 1 min to mix the contents thoroughly. Allow to settle for some time. If the

emulsion does not break, add 3-4 drops of glacial acetic acid, but do not shake. The two layers will separate. When the water layer is perfectly clear, pipette out with an aspirator bulb as much water as possible without sucking the chloroform layer and store it in a 100 ml; volumetric flask. Again add 25 ml of water and repeat the procedure 3 times more to extract all the glycerol. Combine all water extracts; make to 100 ml and mix thoroughly. Keep this extract for estimation of glycerol.

Add chloroform to the mark, taking off as much as the residual water as possible. Stopper the flask and mix the contents thoroughly. Allow the flasks to stand for 15 min.

Pipette out 25 ml aliquot from chloroform extract into 500 ml glass stoppered flask. Prepare a blank containing 25 ml of chloroform only. Pipette out 25 ml periodic acid reagent solution into each sample and also the blank. Stopper the flasks and mix the contents by gentle swirling. Allow the flasks to stand for 1-1.5 h during which the contents of all the flasks should be frequently shaken to mix the sample with the reagent. At the end 1.5 h, add 15 ml of 15% KI solution; wait for 1 min and add 100 ml water. Shake gently and titrate immediately with 0.1N sodium thiosulphate solution, using starch solution as indicator.

$$\text{Monoglyceride, \% by mass} = \frac{(B-S)\times N\times M\times 4}{W\times 20}$$

Where,

B= titre of periodic acid reagent in chloroform blank;

S=titre of sample for monoglyceride;

N=normality of sodium thiosulphate solution;

M= molecular mass of monoglyceride; and

W= mass of sample taken for the test.

Glycerol

Pipette out 25 ml aliquot from the combined aqueous extract. Prepare a blank containing 25 ml distilled water only. Pipette out 25 ml of periodic acid reagent solution into each flask. Stopper the flasks and mix the contents by gentle swirling. Allow the flasks to stand for 30 min. At the end of 30 min, add 15 ml of 15% KI solution; wait for 1 min and add 100 ml water, shake gently and titrate immediately with 0.1N sodium thiosulphate solution using starch solution as indicator.

$$\text{Glycerol, \% by mass} = \frac{(B-S)\times N\times 2.302\times 4}{M}$$

Where,

B= titre of periodic acid reagent solution in water blank;

S=titre of sample for monoglyceride;

N=normality of sodium thiosulphate solution; and

W= mass of sample taken for the test.

xii. Polyglycerol Esters of Fatty Acids

Polyglycerol esters of fatty acids are the esters of fatty acids of food fats with a mixture of polyglycerols. These are being permitted for use as release agents in bakery industry and in chocolate manufacture.

Test for Fatty Acids

Reflux 1 g of sample with 15 ml of 0.5N ethanolic KOH for 1h. Add 15 ml of water, acidify with about6 ml of dilute HCl. Oil drops or a white to yellowish white solid is produced which is soluble in 5 ml of hexane. Remove the hexane layer. Extract again with 5 ml of hexane and again remove the hexane layer. Use the aqueous layer for detection of acids other than fatty acids.

Detection of acids other than fatty acids

Acetic Acid : Transfer about 5 ml of the aqueous layer into a dish. Add excess calcium carbonate and evaporate until dry. Transfer the major part of the residue into a glass tube. Place a filter paper, moistened with reagent for acetone (a saturated solution of o-nitrobenzaldehyde in sodium hydroxide, freshly prepared) on the top of the tube. Heat and observe the yellow colour changes into greenish blue by reaction of the reagent for acetone, with the calcium acetate formed.

Succinic Acid: Transfer one drop of the aqueous layer and a drop of a 0.5% solution of ammonium chloride and several mg of zinc powder into a micro test tube. The mouth of the tube is covered with a disk of filter paper moistened with a solution, in benzene of 5% p-dimethylaminobenzaldehyde and 20% trichloroacetic acid. The bottom of the test tube is heated vigorously with a micro flame for about 1 min. Depending on the amount of succinic acid or succinimide, red-violet or pink stain appears on the paper.

Fumaric Acid : transfer 1 ml of the aqueous layer with 1 ml of 2N sodium carbonate into a test tube. Add 2 or 3 drops of 0.1N potassium permanganate. The solution is promptly discoloured.

Tartaric Acid : Evaporate about 5 ml of the aqueous layer in a porcelain dish until dry. Add 2 ml of conc sulphuric acid containing 0.5% pyragallol and heat on a steam bath. An intense violet colour is produced.

Citric Acid : To 3 ml of the aqueous layer add a few drops of 1% potassium permanganate and warm until the colour has disappeared. Then add an excess of bromine solution. A white precipitate (pentabromacetone) is formed immediately on cooling. Evaporate 1 ml of the aqueous layer resulting from test in a porcelain dish add 1 ml of a mixture of 1 volume acetic anahydride and 5 volume of pyridine into the warm dish. A violet colour is produced. Tartaric acid produces a green colour.

Lactic Acid : Transfer 0.2 ml of the aqueous layer add 2 ml of conc sulphuric acid into a test tube and place for 2 min in boiling water. Cool and add 1-2 drops of a 5% guaiacol solution in ethanol. A red colour is immediately produced.

Polyols Content by GC

Polyglycerol esters are saponified with alcoholic KOH solution and the fatty acids

removed by extraction. The polyols are converted to trimethyl silyl derivatives and analyzed by GC.

Procedure

Weigh about 0.5 g of sample and reflux with 20 ml of ethanolic 1N KOH solution for 2 h. Reduce the volume of ethanol by evaporation at 45-50°C in a stream of nitrogen. Add 10 ml of water and convert the soaps to free fatty acids by acidifying with conc HCl. Extract the fatty acids from the aqueous phase with successive 20 ml portions of light petroleum extracts with water (20 ml) and combine the wash with the aqueous phase. Adjust the aqueous polyol solution to pH 7.0 with aqueous KOH solution with the aid of a pH meter. Evaporate to a small volume (2-3 ml) under reduced pressure and extract three times with 30 ml of boiling ethanol. Filter off any residue and evaporate the ethanol under reduced pressure to yield a viscous liquid sample of polyols. Dissolve a 0.1 g sample of polyol in 0.5 ml of warm pyridine (previously dried over KOH) in a 10 ml capped phial. Add 0.2 ml hexamethyl disilazane, shake, add 0.2 ml trimethyl chlorosilane and shake again. Stand on a warm plate (about 80°C) for 3-5 min. Check that white fumes are present indicating an excess of reagent.

GLC

GC with a FID and a column (1.5 m×4 mm I.D.) packed with 3% OVI on diatomite CO (100=-200 mesh) or on Gas chrom 0 (100-120 mesh). Operating conditions are as follows:

- Oven temperature : Programmed from 90-330°C at 4-6°C/min
- N2 carrier gas flow rate : 86 ml/min
- Injection temperature : 275°C
- Detector temperature : 350°C

Inject a 2 ml sample of TMS derivative of polyols.

Total Fatty Acid Esters

Take 20 g of a homogeneous sample of emulsifier in a 1 litre Erlenmeyer flask. Dissolve in 100 ml chloroform. Add to it 200 ml methanol and mix the blend with a magnetic stirrer. After mixing, add another 100 ml chloroform and continue the mixing. Transfer the solution into 1 litre separating funnel. Wash the Erlenmeyer flask with 30 ml chloroform and transfer to the separating funnel. Then add 180 ml water and mix the contents of the separating funnel. Let the two phases separate from each other. Extract chloroform phase two times with a 10 ml mixture of 9:10-water:methanol. After separating, the water: methanol mix is combined with the original water phase. Wash the combined water phase, once in a 500 ml separating funnel with 50 ml chloroform.

Transfer the chloroform phase which contains the fatty acid ester to a 500 ml round bottomed flask and distil off the solvent in rotary vacuum drier. After the chloroform has been removed, treat the flask further for 20 min. Dry the flask for 20 min in oven at 105°C and after cooling in a desiccator weigh.

$$\text{Total fatty acid ester, \% by mass} = \frac{X \times 100}{M}$$

Where,

M = mass, in g, of sample and

X= mass, in g, of residue after drying.

Total Glycerol

Weigh 1 g of sample in a 300 ml Erlenmeyer flask. Add 25 ml of 0.5N alcoholic KOH solution and saponify the sample for 30 min by boiling under reflux. After saponification neutralize the sample with an equivalent amount of 0.5N HCl. Further add o.5 ml of HCL. Distil off the alcohol in the rotating evaporator. Transfer the contents of the flask to a 500 ml separating funnel with 100 ml hexane and 25 ml distilled water. Shake, after the separation, transfer the water phase to a 200 ml flask fitted with NS 29. Wash the hexane phase two times with 25 ml distilled water each time. Both the water phases are united with the first phase in the flask. Distil the water in the rotating evaporator. Dissolve the remaining glycerol in 2-propanol and filter through a Whatman filter paper No. 41 into a 100 ml flask fitted with NS 29. Wash the flask and filters with 2-propanol in order to transfer the sample quantitatively. After distilling off the 2-propanol in the rotating evaporator on a water bath, apply full vacuum from the water jet pump for 20 min followed by drying for 20 min in the oven and finally cool in the desiccator. Weigh.

$$\text{Total glycerol, \% by mass} = \frac{B \times 100}{A}$$

Where,

A= mass, in g, of the sample, and

B=mass, in g, of the dried polyalcohol phase.

Free Glycerol

Take 20 g of a homogeneous sample of emulsifier in a 1 litre Erlenmeyer flask. Dissolve in 100 ml chloroform. Add to it 200 ml methanol and mix the blend with a magnetic stirrer. After mixing, add another 100 ml chloroform and continue the mixing. Transfer the solution into 1 litre separating funnel. Wash the Erlenmeyer flask with 30 ml chloroform and transfer to the separating funnel. Then add 180 ml water and mix the contents of the separating funnel. Let the two phases separate from each other. Extract chloroform phase two times with a 10 ml mixture of 9:10-water:methanol. After separating, the water: methanol mix is combined with the original water phase. Wash the combined water phase, once in a 500 ml separating funnel with 50 ml chloroform.

Transfer the water phase into a weighed 500 ml round bottom flask and distil off the solvent in a rotating evaporator under vacuum. As soon as the chloroform and methanol has been distilled off, treat the flask further full vacuum in the rotating evaporator. Dry the flask for 20 min in an oven at 105°C and after cooling in a desiccator, weigh.

Free glycerol, % by mass = $\frac{M1 \times 100}{M}$

Where,

M= mass, in g, of the sample, and

M1=mass, in g, of free glycerol remained in flask after drying.

15.7 PRESERVATIVES

i. Sorbic Acid

Sorbic acid, food grade used as a food additive is permitted under the PFA rules (1955).

Purity

Weigh 1.5 g of the material, previously dried in a vacuum desiccator over concentrated sulphuric acid for 24 h. Dissolve in about 25 ml of ethanol, titrate with 1N NaOH using phenolphthalein as indicator. 1 ml of 1N NaOH = 0.1121 g of sorbic acid.

Aldehydes

Prepare a saturated aqueous solution of the material. In a test tube to 1 ml of this solution add 0.5 ml of Schiff's reagent (aqueous solution of 0.125 g of crystalline rose aniline chlorohydrate in 1000 ml and decolourized with sulphuric acid) allow to stand for 10 min. Compare the colour with that produced by 1 ml of formaldehyde solution corresponding to 0.1 % aldehydes, with the same amount of Schiff's reagent under the same conditions. The colour produced in the test solution shall not be more intense than that in the formaldehyde solution.

Test for Stability

Place 10 g of the material in a test tube and heat in an oil bath at 105°C for 90 min. The test tube should be immersed in the oil bath so that the upper level of the material is 2 cm below the surface of the oil. The material shall be taken as having passed the test if no discolouration occurs.

ii. Sodium Propionate

Sodium propionate is an anti-roping agent and a mould inhibitor. It is permitted for bread and certain foods under the PFA rules.

Purity

Weigh accurately about 250 mg of the sample and dissolve it in 40 ml of glacial acetic acid, warming if necessary to effect solution. Cool to room temperature; add 2 drops of methylrosaniline chloride (dissolve 100 mg of methylrosaniline chloride in 10 ml glacial acetic acid). Titrate with perchloric acid. Perform blank and make necessary correction. Each ml of 0.1N perchloric acid is equivalent to 9.606 mg of $C_3H_5NaO_2$.

iii. Calcium Propionate

Sodium propionate is an anti-roping agent and a mould inhibitor. It is permitted for bread and certain foods under the PFA rules.

Purity

Dissolve about 400 mg of the sample, accurately weighed, in 150 ml of water, add 15 ml of sodium hydroxide and 300 mg of hydroxynaphthol blue indicator. Titrate with disodium ethylenediamine tetraacetate (EDTA) until the solution is clear blue in colour. Each ml of 0.05M Na_2 EDTA (16.81 g/L) is equivalent to 9.311 mg of $C_6H_{10}O_4Ca$.

iv. Sulphur Dioxide

Use of sulphur dioxide, food grade is permitted in certain foods under the PFA rules

Purity

An accurately measured volume of the gas shall be absorbed in alkaline solution and determined by iodometric titration.

Non-Volatile Residue

Collected 300 ml of liquid sulphur dioxide in a 500 ml Erlenmeyer flask and stopper the flask lightly with a cotton plug. Evaporate under a well-ventilated hood. When evaporation shall be complete, only a trace of residue shall be observed in the flask.

v. Silver Leaf

Silver leaf is used extensively in India for edible purposes and as a coating on sweets, pan and other eatables. Very high purity of silver has been recommended in order to avoid possibilities of contamination due to harmful elements.

Purity as per IS:2113-2002

Weigh 1 g of sample accurately into 250 ml beaker. Add 15 ml of dilute nitric acid (1:1), cover the beaker with watch glass and heat gently on the hot plate till the sample is completely dissolved. Expel all the nitrous fumes. Remove the beaker from the hot plate and remove the watch glass by washing with a fine jet avoid spurting. Dilute the content to about 100 ml with distilled water. Add 20 ml of HCl to it slowly with continuous stirring avoiding any loss of AgCl while stirring. Cover the beaker with watch glass and keep the beaker on hot plate, avoiding boiling. After 1 h check with few drops of dilute HCl for the complete precipitation of silver. Keep on the hot plate at least for 2 h) preferably over night in a dark place).

Remove the watch glass from the beaker while washing very gently with a fine jet of reagent grade water, wash down the walls of the beaker with fine jet, and filter through previously cleaned, dried and weighed sintered glass crucible using vacuum pump. Adjust the rate of filtration, by adjusting the pressure of the pump so that it is neither too fast nor too slow. Wash the precipitate 2-3 times with dilute nitric acid (3%) and then 5-6 times with distilled water.

Dry the sintered glass crucibles with silver chloride precipitate in oven at 140°C for at least 1 h. Cool the crucibles in desiccator to room temperature and weigh after 1 h. Repeat the procedure till constant weight is obtained.

$$\text{Silver (as Ag), part per thousand} = \frac{A \times 0.7526 \times 1000}{B}$$

Where,

A= mass, in g, of silver chloride, and

B=mass, in g, of the sample.

15.8 FLAVOURING AGENT

VANILLA (*Vanilla fragrans*)

Vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid by HPLC method

Reagents

- Ethanol (96% v/v)
- Methanol
- Dilute phosphoric acid (0.01M)
- Mobile phase : 0.0M Phosphoric acid & methanol (75:25)
- Prepare standard solutions like Vanilin (0.1 g/L), Vanillic acid (0.008 g/L), 4-hydroxybenzaldehyde (0.0008 g/L) and 4-hydroxybenzoic acid (0.002 g/L) in mobile phase.
- Internal standard solution (acetyl salicylic acid, 0.6 g/L)

Procedure

Grind the sample and homogenize it thoroughly. Weigh 20 g of the prepared sample in thimble and extract the test portion in the Soxhlet extractor with about 200 ml of ethanol for 16 h. Make 200 ml of extract in a volumetric flask and make one-in-ten dilution of this solution with mobile phase. Conduct the analysis by HPLC under the following conditions using the mobile phase with a flow rate of 1ml/min.

- UV detector : wavelength 254 nm
- Injection volume :10-100 ml
- Temperature : Ambient
- Column : C18 bonded silica of 5-10 particle size

Calibration

Inject the standard and internal standard separately and then as a mixture. Adapt

the operating conditions so that the resolution factors of the peaks of all the compounds are at least equal to 1. Inject the prepared sample after filtering through a membrane filter (0.45 mm).

Check Your Progress - 4

1. Explain the method for the determination of purity of lecithin and sodium alginate.

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2. What method are used to determine the degree of substitution and Na cl % by mass in CMC?

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3. Name the technique used for the identification of sugers in guargum and Tragacanth gum.

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   ……..…….....

4. How the purity of common salt is determind?

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5. How monoglycerides % by mass is determined in glyeerol monostearate?

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15.9 LET US SUM UP

Non milk food ingredients have same importance in the quality of dairy products as the raw milk or its ingredients. The knowledge of standard specifications of the

ingredients and their test method help us in assaying their purity and detect the adultration or impurities present in these ingredients.

15.10 KEY WORDS

Annatto colour : Natural colour extracted from the seeds of Bixa orellana plant

Saffron : Flakes or powder of petals of saffron flower.

Pigment : Natural colour produced either in flowers or other organs of plants

Carmel : Brown colour produced by burning of sugar

Synthetic food colour : Coloured chemical substance which are permitted to be used as clouring matter in food.

Acidulants : Acids used to increase the acidity of food material.

Specific rotation : Sugars are optically active i.e. they rotate the plane of polarized light. The extent of rotation is known a specific rotation.

Invert sugar : Sucrose in solution rotate the plane of polarized light to the left direction (anti close wise). On its hydrolysis to fructose and glucose, the resultant solution rotate the light to right direction (cloke wise). Therefore, there is an inversion in the direction of rotation hence the hydrolysis is clled inversion and the sugars obtained are called invert sugars.

Reducing Sugar : Those sugars which reduce the fehling solution are called the reducing sugars. In this process the sugar is oxidized.

Antioxident : Those substance which prevent the oxidation of lipids are called antioxidants. Ascorbic acid and tocopharel or vitamin E are natural antioxidants present in food.

15.11 SOME USEFUL BOOKS

BIS (2006) Food Additives. Bureau of Indian Standards, Manak Bhavan, New Delhi

PFA Act (2006), Ministry of Health, Government of India, New Delhi

General Standard for Food Additives, Codex Alimentarius Commission, CAC/STAN 192-1995, Rev. 6 (2005).

Person's Composition and Analysis of Foods (edited by R.S. Kirk and R. Sawyer) 9th Edn., Addison Wesley Publishing Company

15.12 ANSWERS TO CHECK YOUR PROGRESS

Your answers should include the following points:

Check Your Progress – 1

1. Purity of â carotein is determined through extinction of its solution in cyloxanane while the purity of annatto colour is measured by measuring the absorbance of it solution in chloroform.

2. Heating at 60^{o} C at a pressure of 50 mm Hg.

3. 3 and 4 respectively.

4. Through digestion of a known weight and then its distillation and titration of the distillate against standard NaOH solution.

5. Using paper chromatography.

6. Determining dry mass by heating at 105^{o} C and optical density of its solution at 428 nm.

Check Your Progress – 2

1. By titration of a solution of known strength against standard alkali solution.

2. 20^{o} C

3. (x) = 100a/LC in which (x) specific rotation at 20^{o} C

   a = degree of rotation

   L = Length of tube in dm

   C = concentration of substance in g/100 ml solution.

4. i. Fehling solution A 68.28g Cusoy $5H_2$ C/1000ml & distilled water

   ii. Felling solution â (346g of sodium potassium tartrate and 100g NaOH/ 1000 ml water)

   iii. Standard dextrose solution of appropriate concentrate

   iv. Methylene blue indicator

5. Through it hydrolysis with the addition of concentrated Hcl and heating the solution at 60-70^{o} C.

6. To check its purity as this ratio is constant in honey.

Check Your Progress - 3

1. By thin layer chromatography (TLC).

2. Heating the sample at 90° C for 6 hours, then treating the dried sample with acetone and bismuth nitrate and determining the mass of precipitate thus obtained.

3. (x) = 100a/LC in which (x) specific rotation at 20° C

   a = degree of rotation

   L = Length of tube in dm

   C = concentration of substance in g/100 ml solution.

4. i. Fehling solution A 68.28g Cusoy $5H_2$ C/1000ml & distilled water

   v. Felling solution â (346g of sodium potassium tartrate and 100g NaOH/ 1000 ml water)

   vi. Standard dextrose solution of appropriate concentrate

   vii. Methylene blue indicator

4. Through it hydrolysis with the addition of concentrated Hcl and heating the solution at 60-70° C.

5. To check its purity as this ratio is constant in honey.

Check Your Progress – 4

1. Mass of an acetone washed and dried on 105° C reside is determined for the purity of lecithen while for sodium alginate sample dried at 105° C for 4 hours and taken in Hcl is refluxed then after adding NaoH and butanol again it is refluxed for 2h at 145° C after adding barium chloride the mixture is titrated against standard Hcl to find out the volume of 0.25 N NaoH utilized by the stabilizer each ml of 0.25 N NaoN = 27.75 mg of the stabilizer.

2. Degree of substitution is determined by potentiometer i.e. titration of solution of CMC in glacial acid (500 mg/80mg glacial acitic acid) against perchloric acid using pH meter while Nacl content is determined by titrating an acidic solution of the sample against standard silver nitrate solution.

3. Thin layer chromatography (TLC).

4. By titrating the solution of the sample against standard silver nitrate solution using potassium chromate as indicator.

5. Chloroform extract of the sample is titrated again standard sodium Thiosulphate solution after adding execss potassium iodide solution. Starch is used as an indicator.