IS 12711 (1989): Bakery products - Methods of analysis [FAD 16: Foodgrains, Starches and Ready to Eat Foods]

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IS 12711 : 1989
(Reaffirmed 1994)

Indian Standard

BAKERY PRODUCTS — METHODS OF ANALYSIS

भारतीय मानक

बेकरी उत्पाद — विश्लेषण पद्धतियाँ

(First Reprint JUNE 1998)

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

April 1990

Price Group 7

AMENDMENT NO. 1 JULY 1993
TO
IS 12711 : 1989 BAKERY PRODUCTS — METHODS OF ANALYSIS

(*Page* 3, *clause* 10.3, *sentences* 1 *and* 2) — Substitute the following for the existing:

' Weigh accurately about 5 g of the material in a suitable thimble and dry for 2 hours at 100± 2°C. Place the thimble in the Soxhlet extraction apparatus and extract with the solvent for about 8 hours with speed of distillation not less than 5 drops per second.'

(*Page* 3, *clause* 11.3):

a) *Line* 1 — Substitute '5' *for* '10 to 30 g'

b) *Line* 7 — Add the following after the words '8 hours':

'with speed of distillation not less than 5 drops per second'.

(FAD 15)

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Bakery and Confectionery Industry Sectional Committee, AFDC 31

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards on 2 June 1989, after the draft finalized by the Bakery and Confectionery Industry Sectional Committee had been approved by the Food and Agriculture Division Council.

A variety of bakery products having varied composition, palate and nutritive value are produced in our country. At present, a number of methods are employed in assessing their composition, functional characteristics and for detection and quantification of permissible and non-permissible additives. This has led to variations in results obtained in different laboratories and often makes interpretation of results and enforcement of quality standards rather difficult. In order to enforce strict quality control, uniform and reproducible methods of evaluation and analysis are essential. This standard is being prepared with a view to provide uniform and standardized methods of analysis for the benefit of the bakery industry, quality control laboratories and research institutions to ensure uniformity and reproducibility of results all over the country.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

BAKERY PRODUCTS — METHODS OF ANALYSIS

1 SCOPE

1.1 This standard prescribes the methods of analysis of bakery products.

2 REFERENCE

2.1 The following Indian Standard is a necessary adjunct to this standard:

IS No.	*Title*
IS 1070 : 1977	Specification for water for general laboratory use (*second revision*)

3 QUALITY OF REAGENTS

3.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 :1977) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect test results.

4 PREPARATION OF THE SAMPLE

4.1 In case of powdered or granular substances, mix the contents of a whole pack and, if necessary, further grind in a clean and dry mortar to convert it into homogenous powder. Store the ground sample in a clean and dry air-tight glass container.

4.2 For low moisture crisp products, such as biscuits, cookies and rusks, etc, break the contents of the whole pack into small pieces and subsequently grind the pieces either in an electrically driven, clean, dry blender or in a clean and dry mortar to a near homogenous powder. Store the powdered material in a dry air-tight glass container.

4.3 For semi-moist products, such as, cakes, bread, buns, etc, cut the contents of pack into small pieces with the help of clean dry scissors or a sharp-edged knife and further grind in an electrically driven dry blender taking care that the sample temperature does not rise above 45°C in the entire operation. In the case of packs above 400 g, such as bread loaves, slice them uniformly into thin slices with the help of a sharp-edged knife and take two slices from the centre and two from each end leaving the outermost end slices and proceed as described above.

5 DETERMINATION OF MOISTURE CONTENT

5.1 Apparatus

5.1.1 *Moisture Dish*

Made of porcelain, silica, glass, aluminium or stainless steel.

5.1.2 *Oven*

Electric, maintained at 105 ± 2°C.

5.1.3 *Desiccator*

5.2 Procedure

Weigh accurately about 5 g of the prepared sample in the moisture dish, previously dried in the oven at 105°C and weighed. Place the dish in the oven maintained at 105 ± 2°C for 4 hours. Cool in the desiccator and weigh. Repeat the process of drying, cooling and weighing at 30-minute intervals until the difference between two consecutive weighings is less than one mg. Record the lowest mass.

5.3 Calculation

$$\text{Moisture, percent by mass} = \frac{100\,(M_1 - M_2)}{M_1 - M}$$

where

M_1 = mass, in g, of the dish with the material before drying;

M_2 = mass, in g, of the dish with the material after drying to constant mass; and

M = mass, in g, of the empty dish.

6 DETERMINATION OF TOTAL ASH

6.1 Apparatus

6.1.1 *Dish*

Silica.

6.1.2 *Desiccator*

6.1.3 *Muffle Furnace*

6.2 Procedure

Weigh accurately about 5 g of the prepared sample in a tared, clean and dry silica dish. Ignite the material in the dish with the flame of a suitable burner for about one hour. Complete the ignition by keeping in a muffle furnace at 500 ± 10°C until grey ash results. Cool in

a desiccator and weigh. Repeat the process of igniting, cooling and weighing at one hour intervals until the difference between two successive weighings is less than 1 mg. Note the lowest mass. Preserve this ash for the determination of acid insoluble ash.

6.3 Calculation

$$\text{Total ash (on dry basis), percent by mass} = \frac{(M_2 - M) \times 10\,000}{(M_1 - M)(100 - W)}$$

where

M_2 = mass, in g, of the dish with the ash;

M = mass, in g, of the empty dish;

M_1 = mass, in g, of the dish with the material taken for the test; and

W = percent moisture in the sample.

7 DETERMINATION OF ACID INSOLUBLE ASH

7.1 Apparatus

7.1.1 *Dish*

Silica.

7.1.2 *Water-Bath*

7.1.3 *Desiccator*

7.1.4 *Muffle Furnace*

7.2 Reagents

7.2.1 *Dilute Hydrochloric Acid*

5 N, prepared by diluting 1 volume of concentrated hydrochloric acid to 2·5 volumes with water.

7.3 Procedure

To the ash contained in the silica dish (from **6.2**), add 25 ml of dilute hydrochloric acid, cover with a watch-glass and heat on a water bath for 10 minutes. Allow to cool and filter the contents of the dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid. Keep it in an electric air-oven and heat it till it gets dried. Subsequently, ignite the contents of the dish over a burner till the contents get completely charred. Complete the ignition by transferring the dish to a muffle furnace 550 ± 10 °C until grey or white ash results. Cool the dish in a desiccator and weigh. Heat the dish again at 550 ± 10°C for 30 minutes. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing until the difference between two successive weighings is less than 1 mg. Record the lowest mass.

7.4 Calculation

$$\text{Acid insoluble ash (on dry basis), percent by mass} = \frac{10\,000\,(M_2 - M)}{(M_1 - M)(100 - W)}$$

where

M_2 = mass, in g, of the dish with the acid insoluble ash;

M = mass, in g, of the empty dish;

M_1 = mass, in g, of the dish with the material taken for the test; and

W = percent of moisture content.

8 DETERMINATION OF TOTAL SOLID CONTENT

8.1 This value shall be calculated from the moisture percent as 100 minus the moisture percent. The moisture content shall be determined as described under **5**.

9 DETERMINATION OF VOLUME/MASS RATIO

9.1 Equipment

9.1.1 *Wooden Box*

Large enough to contain a loaf of bread in such a manner that the top surface of the loaf remains about 1·5 cm below the top level of the box when the load is placed over a thin layer of rape seeds or mustard seeds in the box.

9.1.2 *Graduated Cylinder and Glass Beaker*

1 000 ml capacity.

9.1.3 *Rape Seeds (or Mustard Seeds)*

9.1.4 *Weighing Scale*

5 kg capacity.

9.1.5 *Glass Beaker*

1 000 ml capacity.

9.2. Procedure

9.2.1 *Determination of Density of Rape or Mustard Seeds*

Weigh the beaker on the weighing scale. Transfer 500 ml of rape seeds or mustard seeds from the measuring cylinder to the beaker and reweigh. Take the average of three readings and calculate the density of the seeds as follows:

$$\text{Density of seeds } (D), \text{ g/ml} = \frac{(B - A)}{500}$$

where

B = average mass, in g, of the beaker plus 500 ml of rape seeds or mustard seeds; and

A = mass, in g, of the empty beaker.

9.2.2 *Determination of Volume of Loaf*

Weigh the loaf after it is cooled to room temperature and record the mass. Fill the wooden

box with rape or mustard seeds avoiding air pockets and level the top surface of the seeds with a wooden plate. Weigh the box with the seeds. Take three such readings and record the average. Empty out the seeds leaving a thin layer at the bottom of the box. Place the loaf on this layer of seeds and fill the rest of the space in the box with rape or mustard seeds. Level off the surface of the seeds by a wooden plate. Weigh the box again. Take three such readings and record the average.

CAUTION — Do not press the loaf while keeping in the box. It should be placed on the layer of the seeds. The upper surface of the seeds should be levelled off with sides of the box.

NOTE — Testing shall be done without slicing the loaf.

9.3 Calculation

Calculate the volume of the loaf in the following manner and determine the volume/mass ratio:

$$\text{Volume, in ml, of the loaf} = \frac{C - E}{D}$$

where

C = average mass, in g, of the box filled with seeds plus mass of the loaf;

E = average mass, in g, of the box filled with loaf, with seeds in the residual space; and

D = density, in g/ml, of rape or mustard seeds (*see* 9.2.1).

10 DETERMINATION OF FAT

10.1 Apparatus

10.1.1 *Soxhlet Extraction Apparatus*

10.2 Reagent

10.2.1 *Petroleum Ether*

Distilling between 40 and 60°C.

10.3 Procedure

Weigh accurately about 10 to 30 g of the material sufficient to give about 1·0 g of fat in a suitable thimble and dry for 2 hours at 100 ± 2°C. Place the thimble in the Soxhlet extraction apparatus and extract with the solvent for about 16 hours. Dry the extract contained in the Soxhlet flask, the empty mass of which has been previously determined by taring at 95 to 100°C for an hour. Cool in a desiccator and weigh. Continue the alternate drying and weighing at 30 minutes intervals until the loss in mass between two successive weighings is not more than 2 mg. Record the lowest mass. Preserve the fat for the determination of fat acidity.

10.4 Calculation

$$\text{Fat, percent by mass} = \frac{100\,(M_1 - M_2)}{M}$$

where

M_1 = mass, in g, of the Soxhlet flask with the extracted fat;

M_2 = mass, in g, of the empty Soxhlet flask, clean and dry; and

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

11 DETERMINATION OF ACIDITY OF EXTRACTED FAT (AS PERCENT OLEIC ACID)

11.1 Apparatus

11.1.1 *Soxhlet Apparatus*

With 250-ml flat-bottom flask.

11.2 Reagents

11.2.1 *Petroleum Ether*

Boiling point below 80°C.

11.2.2 *Benzene-Alcohol-Phenolphthalein Stock Solution*

To 1 litre of distilled benzene, add 1 litre of alcohol or rectified spirit and 0·4 g of phenolphthalein. Mix the contents well.

11.2.3 *Standard Potassium Hydroxide Solution*

0·05 N.

11.3 Procedure

Weigh accurately about 10 to 30 g of prepared sample and transfer it to the thimble and plug it from the top with extracted cotton and filter paper. Dry the thimble with the contents for two hours at 100°C in an oven. Take the weight of empty dry Soxhlet flask. Extract the fat in the Soxhlet apparatus for 8 hours and evaporate the solvent in the flask on a water bath. Remove the traces of the residual solvent by keeping the flask in the hot air oven at 100°C for about half an hour and weigh.

Cool the flask and add 50 ml of mixed benzene-alcohol-phenolphthalein reagent and titrate the contents to a distinct pink colour with potassium hydroxide solution taken in a 10 ml microburette. If the contents of the flask become cloudy during titration, add another 50 ml of the reagent and continue titration. Make a blank titration of the 50 ml reagent. Subtract from the titre of the fat, the blank titre.

11.4 Calculation

$$\text{Acidity of extracted fat (as oleic acid), percent by mass} = \frac{1{\cdot}41 \times V}{M_1 - M}$$

where

V = volume of 0·05 N potassium hydroxide solution used in titration after subtracting the blank;

M_1 = mass, in g, of Soxhlet flask containing fat; and

M = mass, in g, of empty Soxhlet flask.

12 DETERMINATION OF *p*H OF THE AQUEOUS EXTRACT

12.1 *p*H of aqueous extract of the sample shall be determined either by the *p*H meter with glass electrode or by a suitable *p*H comparator provided with standard colour discs. In case of dispute, however, *p*H shall be determined by the *p*H meter.

12.2 Apparatus

12.2.1 p*H Meter with Glass Electrode or* p*H Comparator*

The latter provided with standard *p*H colour discs for the indicator solution given under **12.3.2.**

12.3 Reagents

12.3.1 *Water*

Use distilled water of *p*H 6 2 to 7·0. Boil it for about 10 minutes and cool to room temperature immediately before use. Redistil the water in an all-glass apparatus if its *p*H does not lie within this range.

12.3.2 *Indicator Solutions*

Universal indicator.

12.4 Procedure

12.4.1 *Preparation of Aqueous Extract of the Material*

Grind to a fine paste about 10 g of the material in a glass mortar. Add 100 ml of water and mix thoroughly. Allow the mixture to stand for about 15 minutes. Filter the mixture and collect the filtrate in another beaker.

12.4.2 *Determination of* p*H of Aqueous Extract*

Determine the *p*H of the solution by the *p*H meter, or by using the *p*H comparator as described in **12.4.2.1.**

12.4.2.1 Clean the two glass tubes of the *p*H comparator with carbon tetrachloride to remove any oily or greasy film on them and allow the tubes to dry. Fill the two tubes with the aqueous extract to the 10-ml mark. Add 5 drops of universal indicator in the *p*H comparator. Place the two tubes in position in the comparator, the one without the indicator being placed behind the colour discs and compare the colour until the nearest colour match is obtained. Record the approximate *p*H of the aqueous extract. Discard the contents of the glass tubes to which indicator had been added. Wash with water and dry. Clean the tube with carbon tetrachloride and dry it. Fill the tube with the aqueous extract to the 10-ml mark, add 5 drops of the universal indicator solution and mix the contents well. Place this tube in position, replace the previous standard colour disc with the one provided for the indicator used and read the exact *p*H of the solution when the nearest colour match is obtained.

13 DETERMINATION OF CRUDE FIBRE

13.1 Reagents

13.1.1 *Petroleum Ether*

13.1.2 *Dilute Sulphuric Acid*

1·25 percent (*m/v*), accurately prepared.

13.1.3 *Sodium Hydroxide Solution*

1·25 percent (*m/v*), accurately prepared.

13.1.4 *Ethyl Alcohol*

95 percent by volume.

13.2 Procedure

Weigh accurately about 2·5 g of the moisture-free sample after determining the total solid content and extract for about one hour with petroleum ether, using a Soxhlet apparatus. Transfer the fat-free material to a one litre flask. Take 200 ml of dilute sulphuric acid in a beaker and bring to boil. Transfer the whole of the boiling acid to the flask containing the fat-free material and immediately connect the flask with a water-cooled reflux condenser and heat so that the contents of the flask begin to boil within one minute.

Rotate the flask frequently, taking care not to allow the material to stick to the sides of the flask, and not to keep the material out of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask, and filter through fine linen (about 18 threads to a centimetre) held in a funnel, and wash with boiling water until the washings are no longer acidic to litmus. Bring some quantity of sodium hydroxide solution to boiling under a reflux condenser. Wash the residue on the linen into the flask with 200 ml of boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15 ml of ethyl alcohol, 95 percent by volume. Dry the Gooch crucible and contents at 105 ± 2°C in an air-oven to constant mass.

Cool and weigh. Incinerate the contents of the Gooch crucible in an electric muffle furnace at 600 ± 20°C until all the carbonaceous matter is burnt. Cool the Gooch crucible containing the ash in a desiccator and weigh.

13.3 Calculation

$$\text{Crude fibre (on dry basis), percent by mass} = \frac{10\,000\,(M_1 - M_2)}{M \times (100 - W)}$$

where

M_1 = mass, in g, of Gooch crucible and contents before ashing;

M_2 = mass, in g, of Gooch crucible containing asbestos and ash;

M = mass, in g, of the material taken for the test; and

W = percent of moisture content.

14 DETERMINATION OF ALCOHOLIC ACIDITY

14.1 Reagents

14.1.1 *Neutral Ethyl Alcohol*

90 percent (*v/v*).

14.1.2 *Standard Sodium Hydroxide Solution*

Approximately 0·05 N.

14.1.3 *Phenolphthalein-Indicator Solution*

60 mg of phenolphthalein dissolved in 100 ml rectified spirit.

14.2 Procedure

Weigh 5 g of sample into a stoppered conical flask and add 50 ml of neutral ethyl alcohol. Stopper, shake and allow to stand for 24 hours, with occasional shaking. Filter the alcoholic extract through a dry filter paper. Titrate the combined alcoholic extract against 0 05 N standard sodium hydroxide solution using phenolphthalein as indicator. Calculate the percentage of alcoholic acidity as sulphuric acid.

14.3 Calculation

$$\text{Alcoholic acidity (as } H_2SO_4\text{) in 90 percent alcohol, percent by mass} = \frac{24{\cdot}52\,A\,N}{M}$$

where

A = volume, in ml, of standard sodium hydroxide solution used in titration;

N = normality of standard sodium hydroxide solution; and

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

15 DETERMINATION OF FRUITS IN FRUIT BREAD/CAKE

15.0 The method determines both dry fruits (raisins, cashewnuts, almonds and walnuts) and preserved fruits (*PETHA* peel. *KARONDA* and cherries) in fruit bread/cakes.

15.1 Equipment

15.1.1 *Bread or Cake Knife*

15.1.2 *Brush*

15.1.3 *Weighing Balance*

15.2 Method

Weigh accurately four cake/bread slices (approximately 100 g). Pick one by one each piece of preserved fruit and dry fruit and wipe them individually with a camel hair brush on a filter paper or clean cloth. Seggregate preserved and dry fruits and weigh each type separately and calculate the percentage of fruits from the mass of fruits picked out separately for preserved fruits and dry fruits.

16 DETERMINATION OF NON-FAT MILK SOLIDS IN MILK BREAD

16.1 Principle

The method is a colorimetric one for estimating non-fat milk solids in milk bread based on the orotic acid (2, 6-dihydroxypyrimidine-4-carboxylic acid) content. The mean orotic acid content of non-fat milk solids is 62·5 mg/100 g (range 48·0-74·5 mg/100 g).

16.2 Apparatus

16.2.1 *Air-Drying Oven*

16.2.2 *Homogenizer*

16.2.3 *Pipettes*

5, 10 and 25/ml.

16.2.4 *Glass Stoppered Test-Tubes*

16.2.5 *Volumetric Flask*

500, 100, 50 and 10 ml capacity.

16.2.6 *Water Bath*

16.2.7 *Colorimeter*

16.3 Reagents

16.3.1 *Zinc Sulphate*

23 percent (*m/v*) solution.

16.3.2 *Potassium Hexacyanoferrate*

15 percent (*m/v*) solution.

16.3.3 *p-Dimethylaminobenzaldehyde (DAB)*

3 percent (*m/v*) in propanol.

16.3.4 *Standard Orotic Acid*

Dissolve 50 mg orotic acid in a mixture of 1 ml of 0·88 ammonia and 10 ml water. Dilute to 500 ml with water. Take 10 ml aliquot and dilute to 100 ml with water. Further dilute 2·5, 5, 10 and 15 ml of this solution to 50 ml to produce solutions containing 2·5, 5, 10 and 15 μg orotic acid per 5 ml.

16.3.5 *Saturated Bromine Water*

16.3.6 *Ascorbic Acid Solution*

10 percent.

16.3.7 *n-Butyl Acetate*

16.3.8 *Anhydrous Sodium Sulphate*

16.4 Procedure

16.4.1 Weigh the bread on receipt accurately, nearest to 0·1 g. Cut the bread into 2-3 mm slices, spread on paper and allow to dry in a warm room overnight so that the bread is crisp and brittle. The sample should be in equilibrium with the atmosphere so that the moisture content remains constant during grinding. Then return quantitatively the air-dried bread to the balance and re-weigh. Grind to pass through a 850 μm IS sieve, mix and transfer to an air-tight container. Determine the total solids by drying 2 g at 130°C for 1 hour.

16.4.2 Weigh 5 g of dried sample into the beaker of a homogenizer, add 100 ml water and mix at the maximum speed for 1 minute. Filter the supernatant liquor through a 15 cm Whatman No. 541 paper rejecting the first 10 ml. 5 ml is required for the determination.

16.4.3 Into a series of glass-stoppered test tubes, add by pipette 5 ml of test solution (containing 2-15 μg orotic acid), 5 ml of each of the standard orotic acid solutions and 5 ml of water to act as the blank. Add to each tube 1·5 ml of saturated bromine water and allow the mixture to stand at room temperature for not more than 5 minutes. As the addition of bromine water is made to the series of tubes, the times will vary slightly between each, the time of reaction is not critical provided it is between 1 and 5 minutes. Add 2 ml of 10 percent ascorbic acid solution to each tube and place the tubes in a water bath at 40°C for 5 minutes. Cool to room temperature, add to each tube 4·0 ml *n*-butyl acetate and shake vigorously for 15 seconds. Transfer the upper separated layers to dry test tubes containing 1 g anhydrous sodium sulphate. Mix gently. Add another gram of anhydrous sodium sulphate. Mix gently and allow to separate. Transfer the clear butyl acetate layer to a 1-cm cell and measure the optical density at 461 to 462 nm against the blank.

16.5 Calculation

Draw a calibration graph of the standard orotic acid solution plotting the optical density on the *X*-axis against the concentration of orotic acid on the *Y*-axis. Determine the orotic acid content in 5 ml of sample extract by interpolation of the colorimeter reading on the calibration graph, and hence the amount in the dry sample. For converting to milk, assume that skim milk powder contains 62·5 mg orotic acid per 100 g.

17 CHARACTERIZATION AND ESTIMATION OF SYNTHETIC DYES

17.1 Apparatus

17.1.1 *Glass Pestle and Mortar*

17.1.2 *Beakers*

250 and 100-ml capacity.

17.1.3 *Chromatographic Chamber*

30 cm × 20 cm × 10 cm.

17.1.4 *Test Tubes*

17.1.5 *Spectrophotometer*

17.1.6 *Water Bath*

17.1.7 *Porcelain Dish*.

17.2 Reagents

17.2.1 2 percent ammonia in 70 percent alcohol.

17.2.2 *White Knitting Wool*

Boil pure white knitting wool in 1 percent sodium hydroxide solution and then in water to remove alkali. Wash repeatedly with distilled water and dry.

17.2.3 *Chromatographic Paper*

Whatman No. 1.

17.2.4 *Hydrochloric Acid (0·1 N)*

8·5 ml of concentrated hydrochloric acid diluted to 1 litre with distilled water.

17.3 Procedure

Thoroughly grind 10 g of the powdered food material with 50 ml of 2 percent ammonia in 70 percent alcohol. Allow to stand for few hours and centrifuge. Pour the clear supernatant liquid in the disc and evaporate on the water bath. Dissolve the residue in 30 ml water acidified with acetic acid.

Add a 20 cm strip of pure white wool to the above solution and boil. When the wool takes up the colour fairly completely take out and wash it with tap water. Transfer the washed wool to a small beaker and boil gently with dilute ammonia (1 : 4). If the colour is stripped by the alcohol, the presence of an acid coal tar dye is indicated. Remove the wool. Make the liquid slightly acidic and boil. Add a fresh piece of wool and continue boiling until all colour is removed.

Extract the dye from the wool again with a small volume of dilute ammonia, filter through small plug of cotton and evaporate to low bulk. (This double stripping technique usually gives a pure product but it is not always necessary. Natural colours may also dye the wool during the first treatment but the colour is not removed by ammonia.) Transfer the solution to a volumetric flask and make the volume to 50 ml with water.

NOTE — Basic dyes can be separated by making the food alkaline with dilute ammonia, boiling with wool and then stripping with dilute acetic acid. At present, all the permitted water soluble colours are acidic in nature and hence an indication of the presence of a basic dye suggests the presence of a non-permitted colour.

17.3.1 *Separation of Colours by Paper Chromatography*

Take Whatman No. 1 filter paper sheet (15 cm × 30 cm) and draw a line parallel to the bottom edge of the sheet and about 2 cm away from it. Pipette 0·5 ml of the extracted dye solution with the help of a graduated pipette and apply it on filter paper sheet in the form of a band on the line.

Prepare 0·1 percent solutions of the permitted water soluble dyes and with the help of capillary tubes, apply spots of all these dyes on the line leaving about 1·5 cm distance between two spots. Care should be taken to ensure that the solution does not spread on the filter paper. Allow the coloured spots to dry and subsequently suspend the paper sheet in the chromatography chamber such that the lower edge of the sheet remains dipped in the solvent placed in a solvent boat. Any of the following solvent systems may be used for the separation of colours but solvent system at (5) has been found to give the best resolution:

1) 1 ml ammonia (sp gr 0·85) + 99 ml water
2) 2·5 percent aqueous sodium chloride
3) 2 percent sodium chloride in 50 percent ethanol
4) Isobutanol : ethanol : water [1 : 2 : 1 (*v*/*v*)]
5) N-butanol : water : acetic acid (20 : 12 : 5)
6) Isobutanol : ethanol : acetic acid (3 : 12 : 5)

Close the chromatographic chamber tightly and allow the solvent to rise. When the solvent front has risen to about 20 cm from the base line, remove the filter paper sheet and allow it to dry at room temperature. Mark coloured bands and carefully cut the coloured strips from the paper chromatogram. Cut coloured strips into small pieces and transfer them to a test tube and add about 1 ml 0·1 N hydrochloric acid (HCl). Allow the colour to extract and decant the coloured extract in a volumetric flask. Repeat the process of extraction and decanting till all the colour is removed from the paper. Make up the volume. Determine absorbance maximum and read the optical density at absorbance maximum against a blank prepared by cutting an equivalent strip of plain portion of the chromatogram and extracting it with 0·1 N HCl exctly as described in case of sample. From the absorbance values, compute the concentration of the dye by referring to the plot of concentration *versus* optical density (*see* **17.3.2**).

17.3.2 *Plotting of Standard Curve*

Prepare 0·1 percent solution of the dye in 0·1 N hydrochloric acid. Take 0·25, 0·50, 0·75, 1·0, 1·25 and 1·5 ml aliquots of this and dilute to 100 ml with 0·1 N hydrochloric acid. Read their absorbance at respective absorbance maxima. Plot absorbance values against the concentration of the dye.

17.3.3 The *Rf* values and absorbance maxima of the permitted water soluble dyes are given below which may be used as a guide in characterization of the dye and in determining their concentration. However, chromatographic *Rf* values are known to vary slightly because of variation in temperature, solvent purity and solvent saturation of the chromatography chamber. It is, therefore, essential that known dyes should be applied along with the sample for comparison of *Rf* values under actual conditions used in the test.

Solvent System	*Rf Values*						*Absorbance Maximum* 1 *nm*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ponceau 4 R	0·95	0·36	0·42	0·29	0·33	0 29	505
Carmoisine	0·61	0·04	0·56	0·51	0·56	0·28	515
Amaranth	0·77	0·06	0·20	0·24	0·19	0·20	520
Erythrosine	0·23	0·00	0·70	1·00	1·00	0·38	525
Fast red E	0·45	0·00	0·60	0·45	0·54	0·60	505
Sunset yellow FCF	0·78	0·26	0·65	0·49	0·45	0·56	480
Tartrazine	1·00	0·26	0·30	0·26	0·18	0·22	430
Indigo carmine	—	0·07	0·30	0·28	0·21	0·27	615
Brilliant blue FCF	—	—	—	—	—	—	630
Green S	1·00	0·88	1·00	0·73	0·57	0·50	635
Green FCF	—	—	—	—	—	—	624

18 DETERMINATION OF SORBIC ACID

18.0 Two methods, namely, colorimetric and spectrophotometric methods have been given. Any of the two can be used.

18.1 Colorimetric Method

18.1.1 *Apparatus*

18.1.1.1 *Steam distillation apparatus*

18.1.1.2 *Volumetric flask*

50-ml and 1-litre capacity.

18.1.1.3 *Pipette*

5 and 20-ml capacity.

18.1.1.4 *Test tubes*

15-ml capacity.

18.1.1.5 *Spectrophotometer with 10-mm matched cells*

18.1.1.6 *Water bath*

18.1.1.7 *Ice bath*

18.1.2 *Reagents*

18.1.2.1 *Sulphuric acid*

2 N and 0·3 N.

18.1.2.2 *Potassium dichromate solution*

147 mg potassium dichromate dissolved in distilled water and diluted to 100 ml.

18.1.2.3 *Thiobarbituric acid solution 0·5 percent*

Dissolve 250 mg thiobarbituric acid in 0·5 N sodium hydroxide solution in a 50-ml volumetric flask by swirling in hot water. Add 20 ml distilled water, neutralize with 3 ml of 1 N hydrochloric acid and dilute to volume with distilled water. This solution should be prepared fresh before experimentation.

18.1.2.4 *Crystalline magnesium sulphate*

$MgSO_4.7H_2O$.

18.1.2.5 *Standard sorbic acid solution*

Accurately weigh 134 mg potassium sorbate (equivalent to 100 mg sorbic acid) and dilute to 1 litre with distilled water. One ml of solution corresponds to 0·1 mg of sorbic acid. This solution is stable for several days when refrigerated.

18.1.3 *Procedure*

18.1.3.1 *Preparation of the sample*

a) *All types of bread and cakes not containing fruits*

Take one or a half loaf of bread or cake and cut it into slices of 2-3 mm thickness. Spread the slices on the paper and let them dry in a warm place or at room temperature until sufficiently crisp and brittle to grind well. Grind entire sample to pass through 850-micron sieve. Mix well and keep in an air-tight container.

b) *Bread and cakes containing raisins and fruits*

Take one or a half loaf of bread or cake and cut it into slices of 2-3 mm thickness. Spread the slices on the paper and let them dry in a warm place or at room temperature until sufficiently crisp. Comminute by passing twice through a food chopper and dry the sample in an oven at 70°C under a pressure of less than 50 mm of mercury.

18.1.3.2 *Test portion*

Weigh 1·5 to 2·0 g prepared sample into distillation tube containing silicon chips. Add 10 ml of 2 N sulphuric acid and 10 g magnesium sulphate. Steam distil the contents, maintaining 20-30 ml volume in distillation tube with small burner. Avoid charring. Collect 100 to 125 ml distillate in 250-ml volumetric flask within 45 minutes. Rinse the condenser with distilled water and dilute the distillate to volume and mix thoroughly.

18.1.3.3 *Determination*

Pipette 2 ml of test portion and 2 ml of distilled water (for blank) into separate 15 ml test tubes. Add 1 ml of 0·3 N sulphuric acid and 1 ml of potassium dichromate solution and heat in boiling water bath exactly for five minutes. Immerse tubes in ice bath and add 2 ml thiobarbituric acid solution. Replace it in boiling water bath and boil it for 10 minutes. Cool and determine optical density of solution at 532 nm against blank using matched 1-cm cells.

18.1.3.4 *Plotting of the calibration curve*

Pipette 5, 10, 15, 20 and 25 ml sorbic acid standard solutions into separate 500-ml volumetric flasks. Dilute each to volume and mix thoroughly and proceed as specified in **18.1.3.3.** Plot the optical density against μg sorbic acid/ml.

18.1.4 *Calculation*

Calculate the sorbic acid content in the sample after reading the corresponding sorbic acid value of the optical density.

$$\text{Percent sorbic acid in the sample} = \frac{125 \times A \times 100}{M}$$

where

A = sorbic acid content obtained from calibration curve, and

M = mass of sample taken.

Percent sodium sorbate = Percent sorbic acid × 1·34.

18.2 Spectrophotometric Method

18.2.1 *Reagents*

18.2.1.1 *Metaphosphoric acid solution*

5 g phosphoric acid dissolved in 250 ml distilled water and diluted to 1 litre with absolute alcohol.

18.2.1.2 *Mixed ethers*

Petroleum ether : anhydrous ether (1 : 1).

18.2.1.3 *Potassium permanganate solution*

15 g potassium permanganate dissolved in distilled water, diluted to 100 ml and filtered through glass wool.

18.2.1.4 *Sorbic acid solution (stocks)*

200 mg of sorbic acid dissolved in 200 ml of mixed ethers.

18.2.1.5 *Working solution*

10 ml stock solution diluted to 200 ml with mixed ether and this solution corresponds to 0·05 mg/ml.

18.2.1.6 *Reference solution*

Shake 100 ml mixed ethers with 10 ml metaphosphoric acid solution and dry supernatant ether fraction with 5 g anhydrous granular sodium sulphate.

18.2.2 *Apparatus*

18.2.2.1 *High speed blender*

18.2.2.2 *Separating funnels*

500-ml.

18.2.2.3 *Volumetric flasks*

18.2.2.4 *Graduated pipettes*

18.2.2.5 *Ultraviolet spectrophotometer*

Provided with a 0·5 mm monochromator with silica cells of 20 mm thickness fitted with ground lids.

18.2.3 *Procedure*

18.2.3.1 *Preparation of the sample*

Proceed as in **18.1.3.1.**

18.2.3.2 *Test portion*

Accurately weigh 10 g prepared sample in high speed blender cup. Add enough metaphosphoric acid solution to yield a total of 100 ml liquid in mixture. Blend for 1 minute and immediately filter through Whatman No. 3 paper. Transfer 10 ml filtrate to 250 ml separating funnel containing 100 ml mixed ethers and shake for 1 minute. Discard aqueous layer and dry the ether extract with 5 g anhydrous sodium sulphate.

18.2.3.3 *Determination*

Place the ether solution in a silica cell with a ground lid of thickness 20 mm and measure the absorbance of this solution at 250 nm with respect to the reference solution in a similar silica cell.

18.2.3.4 *Plotting of the calibration curve*

Into a series of four 100 ml volumetric flasks, add 1, 2, 4 and 6 ml of working standard sorbic acid solution and dilute to volume with mixed ethers. Determine absorbance of the solutions at 250 nm. Plot the absorbance against mg of sorbic acid/ml.

18.2.4 *Calculation*

Calculate the sorbic acid content in the sample after reading the corresponding sorbic acid value from the calibration curve.

Percent sorbic acid in the sample = (mg sorbic acid/g sample) × (1/1 000 mg) × 100 = mg sorbic acid/10

Percent sodium sorbate = percent sorbic acid × 1·34

19 ACETIC ACID, PROPIONIC ACID AND ITS SALTS

19.0 This standard specifies two methods for the determination of acetic acid, propionic acid and its salts, namely, paper and column chromatographic methods. Paper chromatographic method shall be used for qualitative detection and column chromatographic method shall be used for quantitative estimation of acetic acid, propionic acid and its salts.

19.1 Paper Chromatographic Method

19.1.1 *Apparatus*

19.1.1.1 *Chromatographic tank*

19.1.1.2 *Pipettes*

Graduated, 0·1 ml.

19.1.1.3 *Chromatographic paper*

Whatman No. 1, 20 cm × 20 cm sheets.

19.1.1.4 *Steam distillation apparatus*

19.1.1.5 *Beaker*

25-ml capacity.

19.1.2 *Reagents*

19.1.2.1 *Mobile solvent*

Take two parts of acetone. One part of tertiary butyl alcohol, one part of *n*-butyl alcohol and one part of liquid ammonia and mix them. This solvent should always be prepared fresh.

19.1.2.2 *Chromogenic reagent*

Add 200 mg each of methyl red and bromothymol blue to a mixture of 100 ml formalin and 400 ml absolute alcohol. Adjust to *p*H 5·2 with 0·1 N sodium hydroxide.

19.1.2.3 *Sodium hydroxide*

0·1 N and 1 N.

19.1.2.4 *Phosphotungstic acid*

20 percent solution in distilled water.

19.1.2.5 *Crystalline magnesium sulphate*

$MgSO_4.7H_2O$.

19.1.2.6 *Sulphuric acid*

1 N.

19.1.2.7 *Acetic acid and propionic acid standard solution*

Pipette 1 ml of acetic acid/propionic acid into a 100 ml volumetric flask and dilute to volume with distilled water. Pipette 1 ml of this stock solution into 25-ml beaker and neutralize acid with 0·1 N sodium hydroxide using cresol red indicator avoiding excess alkali. Evaporate to 0·3 ml in a water bath.

19.1.2.8 *Congo red indicator paper*

19.1.3 *Procedure*

19.1.3.1 *Sample preparation*

a) *All types of bread not containing fruits*

Take one or a half loaf of bread and cut it into slices of 2 to 3 mm thickness. Spread the slices on the paper and let them dry in warm room until sufficiently crisp and brittle to grind well. Grind entire sample to pass through 850 micron sieve; mix well and keep in air-tight container before proceeding for experimentation.

b) *Bread containing raisins and fruits*

Proceed as in **19.1.3.1** (a) except comminute by passing twice through food chopper instead of grinding and dry air-dried sample in an uncovered dish for 16 hours at 70°C under pressure of less than 50 mm of mercury.

19.1.3.2 *Distillation*

Weigh accurately 10 g of air-dried bread and transfer it to 150 ml distilling flask. Add 40 ml distillation water and 10 ml of 1 N sulphuric acid. Mix thoroughly and add 10 ml of 20 percent phosphotungstic acid solution. Mix the contents well and add 40 g magnesium sulphate. Swirl the contents well and make the solution acidic to congo red paper with 50 percent sulphuric acid. Connect the condenser and steam generator and distil 200 ml in 35 to 40 minutes. Immediately neutralize the distillate using cresol red and 0·1 N sodium hydroxide. Evaporate the solution to 0·5 ml or evaporate just to dryness and then take up in 0·5 ml distilled water.

19.1.3.3 *Paper chromatography*

Take Whatman No. 1 (*see* **19.1.1.3**) unwashed chromatographic paper and rule starting line 2·5 cm from bottom edge with hard pencil. Spot two 1 μl spots with 1 μl pipette on paper 2·5 cm apart from each other, leaving at least 2·5 cm margin, the first spot being of acetic/propionic acid standard solution and the second of unknown sample. Let the paper dry and clip it to a glass rod and suspend it in chromatographic tank with 50 ml of mobile solvent in a trough. Do not saturate the tank with mobile solvent before inserting the paper. Seal the glass cover with cellophane or other suitable tape and let it develop until solvent reaches 2·5 cm from top of paper. Remove the paper from the tank and let it air dry.

19.1.3.4 Spray chromogenic reagent on front side of the paper. Spraying should be uniform and rather heavy but not to the extent that chromogenic reagent runs or drips. Faint yellow spots indicate presence of propionic acid.

19.1.3.5 To intensify acid spots, place paper in the atmosphere of ammonia fumes momentarily (by placing 50 ml ammonium hydroxide in a 2-litre beaker and exposing to fumes by placing each end in beaker momentarily), entire paper immediately turns green.

Remove paper from ammonia fumes, acids gradually appear as red spots and presence of propionic/acetic acid in the sample may be determined by comparing their *Rf* values with that of the standard propionic/acetic acid. Since colours of the acids are not stable, mark the spot with the pencil as soon as they are completely developed.

19.2 Column Chromatographic Method

19.2.1 *Apparatus*

19.2.1.1 *Chromatographic tube*

Approximately 15 mm × 250 mm constricted at lower end to 4 mm internal diameter.

19.2.1.2 *Steam distillation apparatus*

All glass.

19.2.1.3 *Test-tubes*

16 mm × 150 mm.

19.2.1.4 *Pipettes*

19.2.1.5 *Rubber bulb*

19.2.1.6 *Microfunnel*

19.2.2 *Reagents*

19.2.2.1 *Sodium hydroxide*

0·1 N and 1 N.

19.2.2.2 *Phosphotungstic acid solution*

20 percent in distilled water.

19.2.2.3 *Crystalline magnesium sulphate*

$MgSO_4.7H_2O$.

19.2.2.4 *Sulphuric acid*

1 N.

19.2.2.5 *Formic acid*

0·01 N.

19.2.2.6 *Alphamine red R indicator solution*

50 mg of monoammonium salt of 3-(4-anilino-1-naphthylazo)-2, 7-naphthalene disulphonic acid dissolved in 25 ml distilled water.

19.2.2.7 *Ammonium hydroxide*

1 N.

19.2.2.8 *Silicic acid*

100-mesh.

19.2.2.9 *Chloroform*

19.2.2.10 *Butyl alcohol*

19.2.2.11 *Sulphuric acid*

50 percent.

19.2.2.12 *Absolute alcohol*

19.2.2.13 *Butanol in chloroform 1 percent*

Remove alcohol from chloroform by washing 3 times with water. Add 10 ml of *n*-butyl alcohol to 1 litre of washed chloroform in separating funnel, shake vigorously, add 25 ml of distilled water and shake again. Let it stand until the lower layer clears. Drain and discard the upper aqueous layer. Store it in contact with granular sodium sulphate.

19.2.2.14 *Cresol red indicator*

Dissolve 50 mg *o*-cresol-sulphonaphthalene in 20 ml of absolute alcohol, add 1·3 ml of 0·1 N sodium hydroxide and dilute to 50 ml with distilled water. Use 2 drops for each 25 ml of aqueous solution.

19.2.2.15 *Barium hydroxide standard solution*

0·01 N.

19.2.2.16 *Sodium acetate-sodium chloride solution*

Dissolve 12 g of sodium chloride and 25 g of sodium acetate in distilled water and dilute to 500 ml.

19.2.3 *Procedure*

19.2.3.1 *Sample preparation*

As given in **19.1.3.1**.

19.2.3.2 *Distillation*

Weigh accurately 10 g of air-dried sample and transfer it to 150-ml distilling flask. Add 40 ml distilled water and 10 ml of 1 N sulphuric acid, mix thoroughly and add 10 ml of 20 percent phosphotungstic acid solution. Mix the contents well and add 40 g magnesium sulphate. Swirl the contents well and make the solution acidic to congo red paper with 50 percent sulphuric acid. Condenser and stem generator and distil 200 ml in 35-40 minutes. Transfer the distillate to 400-600 ml beaker, add 10 ml 0·01 N formic acid, make alkaline to phenolphthalein with 1 N sodium hydroxide and evaporate to 5 ml. Transfer it into a 25-ml glass stoppered test-tube rinsing the beaker with three portions of distilled water. If insolube matter adheres to the beaker, rinse with 1 N sulphuric acid. Make this solution alkaline to phenolphthalein and evaporate just to dryness by inserting the test-tube in steam-bath.

19.2.3.3 *Chromatographic separation*

a) *Preparation of partition column*

Take 5 g silicic acid in glazed porcelain evaporating dish and add 1 ml of alphamine red R indicator solution and just enough 1 N ammonium hydroxide to give alkaline colour of the indicator (1 drop is enough). Add maximum amount of distilled water that the silicic acid will hold without becoming sticky or agglomerating in the butyl alcohol-chloroform solution (this amount shall be determined for each batch of silicic acid and usually varies from 50 to 75 percent of the weight of silicic acid). Homogenize the mixture thoroughly in a pestle. Add 25 ml of 1 percent butyl alcohol in chloroform and mix to form slurry that pours readily. Pour this slurry into a chromatographic tube containing small cotton plug in the neck of constricted end. To avoid air pockets, tilt the tube slightly while pouring. If air bubbles form while pouring, eliminate by stirring suspension in tube with long glass rod. Clamp the tube vertically in the ring stand. In the tube, insert one hole rubber stopper fitted with glass tube bent to 90° angle and held in place by a bunsen clamp against the pressure to be exerted. Connect bent glass tube to pressure. Adjust pressure to (0·35-0·70 kgf/cm²). 34·5 to 68·9 kPa so that excess solvent is forced through column dropwise.

During removal of excess solvent, gel packs down. As column packs down, particles of gel adhere to the wall of the tube but eventually gel leaves wall of the tube relatively clean. This is the point of optimum density of column, and column is ready for use. Apply pressure until solvent reaches surface of the column. If solvent passes below surface causing drying or cracking of column or if air pockets are present, extrude packing from the tube, reslurry with the solvent and repack the column.

b) *Preparation of standard propionic/acetic acid solution*

Prepare stock solutions of propionic/acetic acid by diluting 5 ml of propionic/acetic acid to 250 ml with distilled water. Pipette 1 ml of stock solution into a 125 ml Erlenmeyer flask and titrate with 0·01 N sodium hydroxide using cresol red as indicator to pink colour persisting for 45 seconds.

Calculate concentration of the acids as follows:

mg acid/ml standard solution = ml 0·01 N sodium hydroxide × normality × F

where

F = 7·41 for propionic acid and 6·01 for acetic acid.

c) *Preparation of known samples*

Pipette 50 ml of standard solution into a 50 ml beaker and just neutralize with 0·01 N sodium hydroxide solution using phenolphthalein and add 10 drops in excess. Evaporate it to dryness on steam bath.

d) *Column separation*

To the dry residue, add 2 ml of 1 percent butyl alcohol in chloroform solution and while stirring with glass rod, add 50 percent sulphuric acid dropwise until the sodium salts are converted to free acids (acid to congo red paper) and add 1 g of anhydrous sodium sulphate. Place a 50 ml graduated cylinder under column as receiver. Decant the supernatant on to column, pouring it slowly down the side of the tube without disturbing level surface of column. Apply pressure until solvent reaches the surface of gel. Wash the beaker with 1 ml solvent and pour it on to column. Apply pressure until solvent just disappears into sodium sulphate layer. Wash the beaker with another 1 ml of solvent, transfer to the column, wash inside of tube with 1 ml of solvent and apply pressure until solvent just disappears into sodium sulphate layer. Fill the tube with the solvent and apply pressure. Once the level reaches the point 2 to 5 mm above the narrowest portion of the construction of tube, record the volume and remove the receiver.

Transfer the elute to a 125 ml Erlenmeyer flask, rinsing the cylinder with three 5 ml portion of distilled water. Add one drop of cresol red indicator and titrate with 0·1 N barium hydroxide solution. As its end point approaches, stopper flask and shake vigorously to completely extract acid from solvent phase. Correct titration for blank as follows. Collect 25 ml of butyl alcohol-chloroform mixture from column before the acid is transferred, add 15 ml boiled and cooled distilled water and titrate as above with 0·01 N barium hydroxide solution.

19.2.4 *Calculation*

Calculate the results for propionic acid and acetic acid to mg/100 g sample.

Propionic acid, mg/100 g = 7·40 × ml 0·01 N barium hydroxide solution

Calcium propionate = propionic acid × 1·256
acetic acid mg/100 g = 6·01 × ml 0·01 N barium hydroxide solution

19.2.5 *Identification of Propionic/Acetic Acid*

Acid separated in butyl alcohol chloroform solution may be further identified by paper chromatograph in **19.1.3.3.**

20 ANTIOXIDANTS

20.1 Propyl Gallate

20.1.1 *Reagents*

20.1.1.1 *Petroleum ether reagent*

Mix 1 volume of 40-60°C boiling-range petroleum ether with three volumes of 60-100°C boiling-range petroleum ether and shake the mixture for 5 minutes with one-tenth its volume of sulphuric acid. Discard acid layer. Wash several times with water, then once with 1 percent sodium hydroxide solution and followed again by water until washings are neutral. Discard washings and distil petroleum ether in an all-glass apparatus.

20.1.1.2 *Ammonium acetate solutions*

1·25, 1·67 and 10·0 percent aqueous solutions.

20.1.1.3 *Ferrous tartrate reagent*

Dissolve 0·10 g $FeSO_4 . 7H_2O$ and 0·50 g Rochelle salt (sodium potassium tartrate) in water and dilute to 100 ml. The reagent should be used within 3 hours of preparation.

20.1.1.4 *Propylgallate standard solution*

50 mg of propylgallate dissolved in water and dilute to 1 litre with water.

20.1.2 *Preparation of Standard Curve*

Place at least 7 aliquots of standard solution, covering range from 50 to 100 mg, in 50 ml conical flasks. Add 2·5 ml 10 percent ammonium acetate to each flask. Dilute to 24 ml with water and pipette 1 ml ferrous tartrate reagent into each flask. Let solutions stand for 3 minutes and measure absorbance at 540 nm against reagent blank prepared by taking 20 ml 1·25 percent ammonium acetate solution 4 ml water and 1 ml ferrous tartrate reagent. Plot propylgallate against absorbance.

20.1.3 *Procedure*

Accurately weigh 50 g powdered sample and shake with 50 ml petroleum ether reagent. Allow the petroleum ether layer to separate and carefully decant the extract in a 250 ml separating funnel. Add another 50 ml petroleum ether reagent to the residue and transfer the petroleum ether extract to a separating funnel. Repeat the process of extraction and decantation five times with 50 ml portions of petroleum ether reagent. To the combined extract, add 20 ml of 1·67 percent ammonium acetate reagent and gently shake. Allow the two layers to separate and drain out aqueous layer into a 100 ml volumetric flask, being careful not to let any petroleum ether drop fall in the flask. Repeat the extraction twice with 20 ml portions of 1·67 percent ammonium acetate solution, combining aqueous layers in the volumetric flask. Finally

extract with 15 ml water. Allow the aqueous layer to separate and collect it along with ammonium acetate washings. Add 2·5 ml of 10 percent ammonium acetate solution to the combined extract and dilute the volume to 100 ml with water. If the solution is turbid, filter through dry rapid filtering paper to remove turbidity.

Pipette 20 ml aliquots into 50 ml glass test tubes. Add 4 ml water and 1 ml ferrous tartrate reagent and mix well. Measure absorbance at 540 nm against reagent blank prepared by taking 20 ml of 1·25 percent ammonium acetate, 4 ml water and 1 ml ferrous tartrate reagent. Calculate amount of propylgallate from the standard curve.

20.2 Butylated Hydroxyanisole (BHA)

BHA is extracted from bakery products with carbon disulphide (CS_2) and determined by gas liquid chromatography (GLC) using flame ionization detection.

20.2.1 *Apparatus*

20.2.1.1 *Gas chromatograph*

A suitable equipment fitted with hydrogen flame ionization detection and integrator-cum-recorder. The suggestive operating conditions are given below, which may be changed according to the availability of instrument, provided standardization is done:

a) *Column* : Glass, 122 cm length and 4 mm I D packed with Apeizon L on Gas Chrom (80-100 Mesh);

OR

Stainless steel, 183 cm length and 4 mm I D packed with QF-1 silicone oil (fluoro-silicone, FS 1265) on Gas Chrom (80-100 mesh).

b) *Temperature* :

Column : 160°C

Injection : 200°C

Detector : 210°C

c) *Gases* :

i) Carrier Gas — Nitrogen to elute BHA in 3-5 minutes.

ii) Fuel Gas and its flow rate — Hydrogen; 40 ml/min Apiezon column and 25 ml/min for QF-1 column.

iii) Air flow rate — 340 ml/min.

Prepare column and column material by washing the inside of column tubes and glass wool with methyl alcohol and drying.

Slowly sprinkle about 50 g chrome into 800 ml beaker filled with carbon tetrachloride. Remove fine particles that remain on surface with vacuum line and trap. Decant carbon tetrachloride and dry at 150°C.

Transfer 20 g dried gas chrome in 500 ml round-bottom flask. Add 100 ml chloroform or methylene dichloride and mix gently.

Dissolve 1 g Apiezon L or 2 g QF-1 in 50 ml chloroform or methylene dichloride transfer to the flask, and mix gently. Evaporate to dryness, using rotary vacuum evaporator.

Carefully plug exit of column with small plug of fibre glass wool and through-hole septum. Apply vacuum to the exit port and slowly add coated support through the other end, tapping very gently to aid compaction. Pack to within 1 cm of area heated by injection port. Plug with fine glass wool and condition for about 3 days at 200°C with slow stream of nitrogen or until steady baseline is obtained.

20.2.1.2 *Chromatographic tube*

25 mm × 200 mm glass tube with small drip tip (4 mm internal diameter, 6 mm outer diameter and 50 mm long) with or without medium porosity fitted disc, with close fitting tamping rod.

20.2.1.3 *Fine glass wool*

Wash with carbon disulphide and dry.

20.2.2 *Reagents*

20.2.2.1 *Carbon disulphide*

Reagent grade, nearly, colourless. If it is distinctly yellow, distil before use.

20.2.2.2 *BHA and di-BHA standard mixture*

0·02 µg/ml each of BHA di-BHA in carbon disulphide. Dissolve 1 mg of BHA in a small amount of carbon disulphide, add 10 ml internal standard solution and dilute to 50 ml with carbon disulphide. Prepare fresh and store in low-actinic glassware.

20.2.2.3 *Internal standard*

0·1 µg di-BHA/µl carbon disulphide (CS_2). Prepare fresh and store in low-actinic glassware.

20.2.2.4 *Dichlorodimethylsilane solution*

5 ml diluted to 100 ml with toluene.

20.2.3 *Method*

Grind sample to pass through 850-micron IS Sieve and mix well. Place small plug of glass wool at bottom of chromatographic tube and add 20 g sample to column using tamping rod to pack it firmly without solvent. Put another plug of glass wool at the top. Add three 5 ml

portions of carbon disulphide to column letting each portion sink into the column before adding next. Elute carbon disulphide at a rate of 5ml/minute and collect in a 50 ml graduated cylinder. Continue eluting by adding 10 ml portions of carbon disulphide at a time till 50 ml elute is collected. Rinse tip of the column with small amount of carbon disulphide. Accurately add di-BHA to elute to obtain concentration after evaporation of 0 02 μg di-BHA/μl, final solution. Evaporate elute under gentle stream of nitrogen in a hood at room temperature to small volume (5·0 ml). Accurately dilute evaporated sample to appropriate volume (5 ml). Inject 3 to 9 μl sample, using 10 μl syringe into gas chromatograph. Before and after each series of sample chromatographs, inject 3·0 to 9·0 μl standard mixture, and average standard values for calculations. Measure each pack height in mm.

20.2.4 *Calculation*

Calculate mg/kg antioxidants present, correcting for internal standards as follows :

$$\text{BHA} = (H_x/H_s) \times (C_s/C_x) \times (H_{si}/H_{xi}) \times (C_{xi}/C_{si})$$

where

H_x and H_s = height in mm of sample and standard peaks, respectively;

H_{xi} and H_{si} = heights in mm of internal standard peaks in sample and standard, respectively;

C_x and C_s = concentrations of samples (g/μl) and standard μg/μl, respectively; and

C_{xi} and C_{si} = concentrations (μg/μl) of internal standard in sample and standard solution, respectively.

21 MEASUREMENT OF PEROXIDATION IN STORED BAKERY PRODUCTS

21.0 During storage, fat or shortening used in bakery projects, such as biscuits, cookies cakes, etc, undergo auto-oxidation which is often followed by stored odours. Though there is no foolproof method to determine the exact level of anti-oxidant, peroxide value and TBA value are widely used for estimating peroxidation and rancidity in foods.

21.1 Peroxide Value

21.1.1 *Reagents*

21.1.1.1 *Sodium thiosulphate stock solution*

0·1 N.

Dissolve 24·88 g in 1 litre distilled water. Determine its exact normality by titrating against standard potassium dichromate solution.

21.1.1.2 *Sodium thiosulphate*

0·01 N.

Pipette 25 ml of stock solution in 250-ml volumetric flask and dilute to volume.

21.1.1.3 *Starch solution (1 percent)*

Take 1 g soluble potato starch and add about 5 ml water in 250 ml beaker and mix to form a paste. Add 100 ml distilled water and boil for 5 minutes.

21.1.1.4 *Saturated potassium iodide solution*

Take about 25 ml distilled water in 50 ml conical flask and add about 5 g potassium iodide and shake. If it completely dissolves, add more potassium iodide until undissolved crystals remain at the bottom. Solution should be prepared just before use.

21.1.2 *Procedure*

Take 50 to 100 g powdered sample in a stoppered flask, add 100 ml chloroform and shake for about half an hour. Filter through a dry filter paper and transfer 20 ml aliquots to 250-ml iodine flask. Add 30 ml glacial acetic acid and 1 ml saturated potassium iodide solution. Stopper the flask and keep aside for 5 minutes. Run a blank by taking 20 ml chloroform instead of food extract. After 5 minutes add 50 ml water to each flask and mix the contents well. Add 1 ml starch solution (1 percent) as indicator and immediately titrate against 0·01 N sodium thiosulphate solution. For determining the fat concentration in the extract, evaporate 10 ml aliquots in tared aluminium dishes in an air oven at 80°C to constant mass.

21.1.3 *Calculation*

$$\text{Peroxide value (milliequivalent of oxygen per kg fat)} = \frac{(V_1 - V_2) \times N \times 1\,000}{W}$$

where

V_1 = volume of thiosulphate solution consumed for sample;

V_2 = volume of thiosulphate solution required for blank titration;

N = normality of sodium thiosulphate solution; and

W = weight of fat content in the 20 ml chloroform extract of the sample.

21.2 TBA Value

21.2.1 *Reagents*

21.2.1.1 *Hydrochloric acid (3 N)*

Take 250 ml concentrated hydrochloric acid in 1 litre measuring flask and dilute to volume.

21.2.1.2 *Thiobarbituric acid solution (0·67 percent)*

Dissolve 0·67 g thiobarbituric acid in 100 ml glacial acetic acid by slightly warming, if necessary. Prepare fresh solution daily.

21.2.2 *Procedure*

Take 10 g powdered sample in a 250-ml round-bottomed flask, add 7·5 ml 3 N hydrochloric acid and 75 ml distilled water. Steam distil the contents, collecting 50 ml of the distillate in 10 minutes. Accurately, pipette 20 ml aliquots in 50-ml boiling tubes, add 2 ml of 0·67 percent thiobarbituric acid in glacial acetic acid and heat the tubes in a boiling water bath for 30 minutes. Run a blank experiment by taking 20 ml distilled water in place of sample distillate and treating exactly as in the experiment. Measure the colour intensity at 532 nm against the blank. Calculate the concentration of malonaldehyde from the standard curve.

21.2.2.1 *Standard curve*

Weigh accurately about 250 mg of tetramethoxy propane in a glass curette and trasnsfer the curette to a glass stoppered flask and heat at 50°C for 3 hours. Transfer the contents quantitatively to 1 litre volumetric flask and make up the volume to 1 litre. Pipette 1 to 5 ml aliquots of the solution in 50 ml boiling tubes and make up the volume to 20 ml with water. Add 2 ml of 0·67 percent thiobarbituric acid in glacial acetic acid and heat the tubes in boiling water for 30 minutes. Measure the absorbance at 532 nm against reagent blank prepared by taking 20 ml distilled water in place of malonaldehyde (164 tetramethoxy propane = 72 g malonaldehyde).

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