Practical Manual

Food Additives and Preservatives

B.Tech (Food Technology)

Semester : IV (New)
Course No. FCN - 246

CODEX ALIMENTARIUS
International Food Standards
World Health Organization
Food and Agriculture Organization of the United Nations

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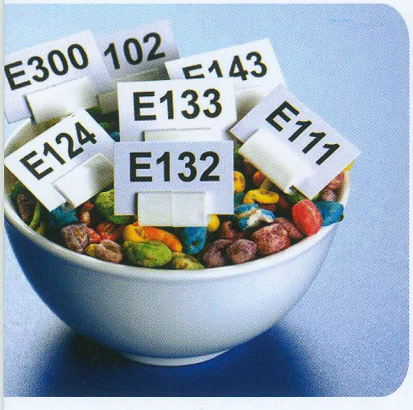

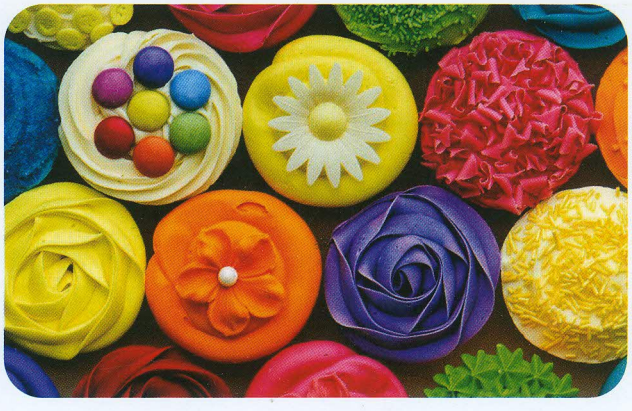

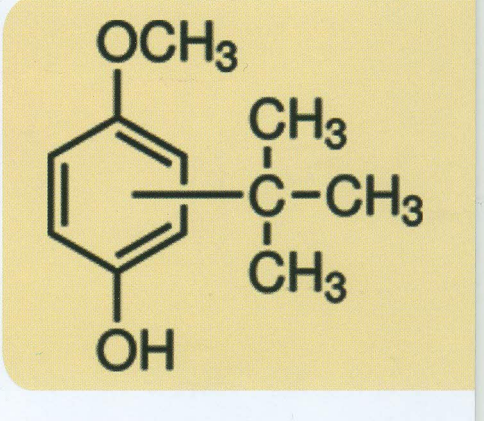

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Certificate

This is to certify that Shri/Ku. _____

Reg.No. _____ has completed the Practical's of Course No. FCN-246 (Food Additives and Preservatives) as per the syllabus for B.Tech (Food Technology) Second Year IVth Semester as prescribed by MCAER, Pune.

Date: / / **Course Teacher**

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EXPERIMENT - 1

Evaluation of GRAS aspect of Food Additives

Introduction :

- Generally recognized as safe (GRAS) is an American Food and Drug Administration (FDA) designation that a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements.
- Generally recognized as safe (GRAS), a system for review and approval of ingredients for addition to food. The GRAS designation is applied when a group of qualified experts agree the product is commonly known to be safe when used as intended.

Process of Safety evaluation of GRAS substances:

The information critical in determining the safety of a GRAS substance and should include at a minimum as follows:

1. Description of the GRAS substance: A review of the physical and chemical characteristics of the GRAS substance includes chemical name(s) (and synonyms), CAS registry number(s), and chemical structure(s) and a description of final product Pressman et al. 13 characteristics includes established food-grade specifications for the principal components, related substances, by-products, impurities and contaminants, and batch analysis results showing compliance with established food-grade specifications.

2. Production process: It includes documentation of good agricultural practice/good manufacturing practice (cGAP/cGMP), detailed process flow diagram for each step of the production process and operation parameters including critical control step(s) in the process, a list of raw materials including specifications and processing aids with food-grade and regulatory compliance documentation, critical control steps involved in the quality control process, description of potential impurities in the final product, and documentation of stability and shelf life.

3. Historical use, regulatory status, and consumer exposure: A review of the history of use and/or natural occurrence of the ingredient in other foods along with an intake or exposure estimate, current regulatory status if any, proposed use and use levels utilized to calculate the EDI of the GRAS substance.

4. Intended effect: It intended function in the food.

5. Analytical methodology: For determining the quantity of the substance in or on food, and any substance formed in or on food because of its use.

6. Review of safety data: Evaluation of the actual use of the product and issues that may contribute to the safety of the product; critical review from the published animal toxicology and clinical literature for safety information on primary components, related substances, secondary metabolites, impurities, and contaminants using relevant data for occurrence and/or levels present, estimated background intake, metabolic fate, and toxicological and pharmacological activity.

7. Safety assessment and GRAS determination: Evaluation of the safety of consumption of the substance under its intended conditions of use including determination of an ADI for the substance as well as other components or contaminants and comparison of this ADI to the EDI of the substance from existing and proposed uses. As long as the EDI is less than (or approximates) the ADI, the substance can be considered safe under its intended conditions of use.

E-Numbers for Different Food Additives

- E numbers are number codes for food additives and are usually found on food labels throughout the European Union. The "E" stands for "Europe" or "European Union". Normally each food additive is assigned a unique number, though occasionally, related additives are given an extension ("a", "b", or "i", "ii") to another E-number.
- The numbering scheme follows that of the International Numbering System (INS) as determined by the Codex Alimentarius committee though only a subset of the INS additives is approved for use in the European Union.

E-Numbers	Food Additives
E100–E199	Colours
E200–E299	Preservatives
E300–E399	Antioxidants, Acidity Regulators
E400–E499	Thickeners, Stabilizers, Emulsifiers
E500–E599	Acidity Regulators, Anti-Caking agents
E600–E699	Flavour Enhancers
E700–E799	Antibiotics
E900–E999	Glazing agents and Sweeteners
E1000–E1599	Additional chemicals

Food additives added in a different food product are as follows:

Sr. No.	Name of the product	Food additive used in food product	E-number
1	Magi	Acidifying agent, flavour enhancer, raising agent colour, acidity regulator	E230, E365, 5000(35), 150D, 500(3)
2	Jam	Thickeners, acidity regulator preservatives	E440(H), E330, E24, E223
3	Ber pulp	Permitted class II preservative	E24
4	Milk powder	Emulsifier	E339
5	Amoul strawberry shaker	Permitted stabilizer, synthetic food colour	E407, E127, E110, E128
6	Fruity	Acidity regulator antioxidant, flavoring substance synthetic food colour	(INS 330) (INS 300)
7	Mango pickle	Acidity regulator, acetic acid	E260
8	Tomato ketchup	Acidity regulator, preservatives, stabilizer	E260, E211,E215
9	Cake	Humectants baking powder, classes preservatives, raising agent, stabilizer. Emulsifier	E420, E202, E500, E415, E450
10	Ice-cream	Permitted emulsifier stabilizer	E471, E407E, E460
11	Kitkat	Antioxidant (Soyalecitins) leaving agent, calcium suitable and improver	500(B)
12	Pizza	Flavour enhancer	E635
13	Crispy pack biscuit	Emulsifier, acidity regulator, dough conditioner, improver vanilla flavour	E471, E320, E270, E223 E1101(ii)
14	Potato chips	Anticaking agent, emulsifier, acidity regulator	E551, E414
15	Ginger-garlic paste	Acidifying agent, emulsifying and stabilizing agent preservatives	E260 AND E330, E413D E211
16	Chewing gum	Humectants, acidity regulator, tale	E420, E422, E330, E533(i)
17	Dairy milk	Emulsifier	E442, E476
18	Bourn vita	Emulsifier raising agent	E322, E471 500 (ii)
19	Kurkure	Antioxidant, acidity regulator	E330
20	Happy-Happy cookies	Dough conditioner, raising agent emulsifier flavour chocolate	E233, E503
21	Chocolate caramel	Emulsifier flavoring substance	E322, E476
22	Mazza	Antioxidant acidity regulator preservatives synthetic food colour	E300, E330, E202, E110
23	Balaji wheels	Acidity regulator	E330
24	Chocó bar	Emulsifying agent permitted emulsifier stabilizer	INS 322, INS 471, INS 407, INS 412
25	Horlicks	Emulsifier acidity regulator	EE 471, INS 501(ii)

Qualitative Test for Presence of Benzoic acid in Food

Materials: Fruit juices

Reagents :

1. 0.5% ferric chloride solution
2. HCL
3. Diethyl ether

1. Ferric Chloride Test:

- Acidify the food product with hydrochloric acid (1+3) and extract with diethyl ether.
- Evaporate the solvent on a hot water bath removing last traces of solvent under a current of air.
- Dissolve the residue in few ml of hot water and add few drops of 0.5% ferric chloride solution. Salmon colour precipitate of ferric benzoate indicates the presence of benzoic acid.

Preparation of Sample:

a. Beverages and liquid products:

- Mix the sample thoroughly and transfer 100 gm of the sample into a 250 volumetric flask, using saturated sodium chloride solution.
- Make alkaline to litmus paper with 10% sodium hydroxide solution and make upto volume with saturated sodium chloride solution.
- Shake thoroughly and let it stand for 2 hrs. Filter the sample and use the filtrate for determination.

b. Sauces and Ketchups:

- Add 15 gm salt to 150 gm of weighed sample and transfer into volumetric flask. Rinse with saturated sodium chloride solution.
- Add 15 gm pulverized sodium chloride and then add 10 ml of 10% sodium hydroxide solution and make upto 500 ml volume with sodium chloride solution.
- Let it stand for 2 hrs with occasional shaking. Filter and use the filtrate for determination.

c. Jams, Jellies, Preservatives and Marmalades:

- Mix 150 gm of sample with 300 ml saturated sodium chloride solution.
- Add 15 gm pulverised sodium chloride. Add 10 ml of 10% sodium hydroxide solution.
- Transfer to 500 ml volumetric flask and dilute to volume with saturated sodium chloride solution.
- Let it stand for 2 hrs with frequent shaking, filter and use the filtrate for determination.

2.Titrimetric Method:

Principle:

The Benzoic acid is separated from a known quantity of the sample by saturating with sodium chloride and then acidifying with dilute hydrochloric acid and extracting with chloroform. The chloroform layer is made mineral acid free and the solvent is removed by evaporation. The residue is dissolved in neutral alcohol and the amount of benzoic acid is determined by titration against standard alkali.

Reagents:

1. Chloroform -distilled
2. Hydrochloric acid (1+3)
3. Sodium hydroxide (10%)
4. Standard sodium hydroxide solution (0.05N)
5. Saturated sodium chloride solution.

Determination Protocol:

1. Pipette 100 ml to 200 ml of the filtrate into a 250 ml separatory funnel.
2. Neutralize to litmus paper using hydrochloric acid (1+3) and add 5 ml excess.
3. Extract carefully with 40, 30, 30 and 20 ml portions of chloroform. Avoid formation of emulsion by shaking gently with rotatory motion. If emulsion forms, break it by stirring chloroform solution with a glass rod after each extraction, but do not drain any of the emulsion with chloroform layer.
4. Transfer the combined chloroform extract in to a separatory funnel and wash it free from mineral acid by shaking gently and rinsing with water.
5. Drain off the water phase. Dry the chloroform layer over anhydrous sodium sulphate and distil off the solvent.
6. Remove the last traces of the solvent under a current of air at room temperature. Dry the residue overnight or until no residue of acetic acid is detected if the product is a ketchup.
7. Dissolve residue in 30-50 ml of alcohol neutralised to phenolphthalein and titrate with 0.05 N sodium hydroxide

Calculate the benzoic acid contents as follows:

$$\textbf{Benzoic acid (ppm)} = \frac{122 \times \text{Titre} \times \text{Dilution} \times 1000 \times \text{ml of 0.05N sodium hydroxide}}{\text{Weight of sample} \times \text{aliquot taken (100 or 200ml of filtrate)}}$$

Observations :

Results:

Qualitative test for presence of sulphurous acids in food

Introduction :

Sulphur dioxide is a widely accepted preservative for many food products such as beverages, squashes, grape resins, dehydrated food products, caramel etc. It is also used for bleaching of sugars and often occurs as a residual component in sugar samples.

Qualitative Test for presence of Sulphur dioxide

- **Reagents**
  Iodine – Barium chloride - Dissolve 3 gm Iodine in water containing 3 gm Potassium Iodide. Add 2 gm Barium Chloride dissolved in water and dilute to 100 ml
- **Apparatus**
  Conical flask with a small bubbler in the form of a small thistle funnel bent twice in the stem so that gases evolved pass through the reagent placed in the funnel.

Procedure:

1. Place 5 gm sample in the flask, add 0.1 gm copper acetate, a piece of marble and 10 ml of conc hydrochloric acid and fit on the bubbler.
2. Allow the acid to act on the marble for 10 min and then heat to boiling. The iodine is decolorized and in the presence of sulphur dioxide a precipitate of barium sulphate settles in the tube. The formation of turbidity is inconclusive as it may be due to other substances such as volatile oils.

Observations :

Results:

Quantitative determination of Benzoic Acid

Principle:

These preservatives are separated by steam distillation, extracted into ether from the acid solution and the ethereal extract is examined by TLC.

Reagents:

i) 50% hydrochloric acid (v/v). Diethyl ether (peroxide free): The absorbance characteristics of this must be checked and if necessary the ether redistilled. Petroleum ether or cyclohexane may be used in place of ether as long as the absorbance in the range 220 - 290 is acceptably low e.g. by use of spectroscopically pure grade.
ii) TLC developing solvent-Ethanol: Ammonia (9:1)
iii) Standard solution: 1% benzoic acid and Sorbic acid in ethyl acetate.
iv) Peroxide- ferric chloride spray regent: Mix equal volume of freshly prepared 2% ferric chloride and 0.5% hydrogen peroxide solution.
v) Thiobarbituric acid spray reagent: 0.2% solution in water of 2- thiobarbituric acid.
vi) Solid magnesium sulphate heptahydrate.
vii) 1M sulphuric Acid.
viii) 1M sodium hydroxide.
ix) TLC plates and silica gel G

Procedure:

1. Place a weighed portion, usually 25 -50 gm of sample in a one lit steam distillation flask, add 100 gm of magnesium sulphate and 100 ml 1M sulphuric acid.
2. Steam and distill as rapidly as possible, collecting about 450 ml in 10 min steam-distillate in the flask containing 10 ml 1M sodium hydroxide solution.
3. Heating the flask containing the sample may result in a coloured or impure distillate. Add 15 ml 1 M sulphuric acid to the distillate and dilute to 500 ml.
4. Extract and aliquot (100 ml) with three or four 25 ml portions of diethyl ether or other solvent.
5. Combine the extracts and wash them with a few ml of water Dry the combined solvent layer over anhydrous sodium sulphate and reduce to 1 ml at the lowest temperature possible. Use of a rotary evaporator is preferable Spot 20 µl or less on the silica gel G TLC plate along with standard solution.
6. Develop for about 10 cm using developing solvent.
7. Air dry the plate and spray with peroxide-ferric chloride reagent. Benzoic acid shows as a mauve coloured spot (Rf 0.5) and Sorbic acid may be distinguished as a yellow coloured spot slightly below it.
8. Further spraying with TBA solution and heating at 100°C for 5 min, Sorbic acid appears as a pink spot a little below benzoic acid (Rf 0.45).

Calculations:

$$\textbf{Rf Value} = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$

Observations :

Results:

EXPERIMENT - 6

Determination of Nitrates and Nitrites in Foods

Introduction :

Sodium and potassium salts of nitrate and nitrite are added mainly to preserve meat and meat products such as cured meat and meat pickles.

Principle:

The sample is clarified with alumina cream and the amount of nitrate present determined by allowing it to diazotise arsenilic acid and coupling the diazonium salt with n-1naphthylethylene diamine. The colour so formed is extracted into n-butanol and the absorbance is measured at 545 nm. An aliquot of the sample is mixed with spongy cadmium in order to reduce any nitrate present and the nitrite so produced is determined in the same way. The amount of nitrate present is then calculated by subtracting the nitrite from the total.

Reagents:

i) Water: This may be distilled or de-ionised but a blank must be carried out to check that it is of satisfactory quality for the preparation of the spongy cadmium.
ii) Alumina Cream: Prepare a saturated solution of potassium aluminium sulphate and add ammonia slowly with stirring until pH is 7.0.
iii) N-naphthylethylene diamine: dihydrochloride 0.1% in distilled water.
iv) Arsenilic acid monohydrate: 0.1% in 5M hydrochloric acid.
v) Buffer pH, 9.6: Prepare 0.7M ammonium chloride (37.45 gm/l) in distilled water and add 0.88 ammonia until the pH is 9.6. Spongy cadmium: Place zinc rods in 20 % aqueous cadmium sulphate solution and leave for 3 or 4 hrs. Separate the precipitated cadmium, wash twice with distilled water and then macerate with water for 2-3 min Activate by shaking with 2M hydrochloric acid and then wash at least 5 times with distilled water, keep the cadmium under distilled water and prepare freshly for each batch of determination.
vi) Standard nitrite solution: Weigh out 0.4783 gm of sodium nitrite and dilute to one L with water. Dilute this 10 times to get 10 mgs /1 of nitrite nitrogen.
vii) n-butanol

Procedure:

1. Mix the sample thoroughly by macerating or homogenizing and weigh 5 gm into a 150 ml beaker. Add 50 ml water and heat to 80°C stirring gently.
2. Maintain at 80°C for 10 min add 20 ml alumina cream and transfer gently to a 100 ml volumetric flask. Cool and dilute to volume with water.
3. Mix and filter through Whatman No.4 filter paper rejecting the first 10 ml of filtrate. The filter paper must be previously washed with at least 100 ml of hot water to remove the small amounts of nitrate that it may have contained

a. Determination of nitrite:

1. Pipette 10 ml of filtrate into a 50 ml volumetric flask, add 2 ml of arsenilic acid solution and mix.
2. Leave for 5 min, and then add 2 ml of napthylethylenediamine solution.
3. Mix and leave for 10 min If the solution is clear, dilute to 50 ml with water and read the absorbance at 538 nm using a 1 cm cell. If the solution is cloudy, transfer to a 100 ml separator, saturate with salt and extract with n-butanol using 20, 15 and then 5 ml.
4. Pass the butanol extracts through a small cotton pledget in a funnel into a dry 50 ml calibrated flask and dilute to volume with n –butanol. Read the absorbance at 545 nm in a 1 cm cell.

b. Determination of nitrate:

1. Pipette 10 ml of filtrate into a small stoppered conical flask.
2. Add 5 ml of buffer solution and one gram of wet cadmium. Stopper the flask and shake for 5 min filter the solution through a washed filter paper into a 50 ml volumetric flask rinsing the cadmium and the filter paper with 5ml water.
3. Determine the nitrite in the filtrate as given above starting at "add 2 ml of arsenilic acid solution".

Preparation of standard curve:

Pipette into a series of 50 ml volumetric flasks dilute standard solution of sodium nitrite containing 2-15 µg of nitrite nitrogen and develop the colour as given in the procedure for nitrite. Read the absorbance and plot standard curve. Repeat the experiment and extract the colour with n-butanol and read the absorbance at 545 nm and also plot a standard curve for this solvent. From the graph calculate the nitrite content before and after reduction and calculate the nitrate content by subtraction.

Observation:

1. Name of samples
2. Absorbency at 545 nm

Calculations :

Results:

Qualitative Test for presence Non -Nutritive Sweeteners

1. Saccharin:

Preparation of the test sample:

a. Non-alcoholic beverages:

1. Add 3 ml hydrochloric acid to about 25 ml of the sample in a separator. If vanillin is present remove it by extraction with several portions of petroleum ether.
2. Discard petroleum ether. Extract with 50, 25 and 25 ml portions of diethyl ether + petroleum ether (1+1) and wash combined extracts with 5 ml water and remove the solvent by evaporation.

b. Semi-solid preparations:

Transfer 25 g of sample to 100 ml volumetric flask with small amount of water and add enough boiling water to make about 75 ml, let mixture stand one hour shaking occasionally. Then add 3 ml acetic acid, mix thoroughly, add slight excess (5 ml) of 20% neutral lead acetate solution, dilute to volume, mix with cold water and let it stand for 20 min and filter. Transfer 50 ml filtrate to separator and proceed as in (a).

1. Phenol sulphuric acid test:

To the residue obtained after removing solvent, add 5 ml of phenol sulphuric acid reagent (pure colourless crystals dissolved in equal weight of sulphuric acid) and heat for 2 hrs at 135-140°C. Dissolve in small amount of hot water and make it alkaline with 10% sodium hydroxide. Magenta or reddish-purple colour develops if saccharin is present.

2. Resorcinol sulphuric acid test:

To the residue add 5 drops of resorcinol-sulphuric acid (1:1) and heat on a low flame until the product turns red. Dissolve in 10 ml of water and make it alkaline using 10% sodium hydroxide solution and add few drops of iodine solution. A green fluorescence is developed if saccharin is present.

2. Cyclamate

Sodium nitrite Test

Procedure:

- Add 2 gm of barium chloride to 100 ml of sample or aqueous extract prepared by grinding sample, adding water to mix uniformally. Add 2-5 gm calcium chloride and shake to dissolve. Make alkaline with 10 % sodium hydroxide, shake, let stand for 2 hrs and filter.
- Acidify filtrate with 10 ml of hydrochloric acid and add 0.2 gm of sodium nitrite Warm the contents on a hot plate. A white precipitate of Barium Sulphate is obtained in the presence of cyclamate.

Note: - Sulphur dioxide interferes with test. Verify its absence by qualitative test.

3. Acesulfame K:-

Qualitative Method (Thin-layer Chromatographic detection of acesulfame Saccharin and cyclamate):

Apparatus:

- UV lamp (360 nm);
- Ion-exchange resin: Amberlite LA-2.

Reagents:

- Polyamide
- 2,7-dichlorofluorosein
- Bromine
- Formic acid
- Ammonia 5%
- Xylol
- Propanol
- Methanol
- Developing solution: Xylol: n-propanol: formic acid:: 5:5:1

Procedure:

- Extract the sweetener from acidified food product with water or take acidified aqueous extract and pass through the ion-exchanger and wash with water. Elute the sweeteners with dilute ammonia solution.
- Evaporate the ammonical solution under vacuum to dryness and take up the residue in 1 ml of 50% methanol (alternatively extract these sweeteners from acidified sample, pH 0.6, with ethyl acetate and use concentrated ethyl acetate for TLC).
- Apply 2-10 µl of sample solution along with standards on TLC plates coated with polyamide. Develop the plate to about 15 cm height with a developing solvent consisting of xylol: n-propanol: formic acid (5:5:1).
- Dry the plates in a current of air and spry with 0.2% solution of dichlorofluoresein and after being dried, examine under UV light. To identify the spots in day light, place the plate in chamber containing bromine and then expose to ammonia vapour. Spots appear on a reddish background.

Observations :

Results:

EXPERIMENT - 8

Identification of colours in food by TLC

Introduction:

Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.

Principle:

In adsorption chromatography mixture is dissolved in solvent, itself poorly adsorbed or applied to the stationery phase this solution contact to the surface of stationary phase. The solutes are more or less strongly adsorbed (held on the surface) thus, remove the solvents. This process is called spotting or striking.

Developing solvent (mobile phase):

100 ml of petrol ether, 11 ml of isopropanol and 5 drops of distilled water. Petroleum ether is volatile and very flammable. Petroleum ether presents a high fire risk. The toxicity of petroleum ether varies according to its composition. Many of the components are of quite low toxicity, but some formulations may contain chemicals that are suspected carcinogens. Avoid ingestion and inhalation. Acetone and isopropanol are highly flammable

Preparation of the TLC chamber:

The developing solvent is placed into a TLC chamber. The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm. The chamber is closed and shaken. It is kept covered so that evaporation doesn't change the composition of the developing solvent mixture. After 15 minutes the chamber will be saturated with the solvent vapor.

Extraction of the leaf pigments:

Using a pestle fresh leaves are grinded in a mortar containing 22 ml of acetone, 3 ml of petroleum ether and a spatula tip-ful of $CaCO_3$. The pigment extract is filtered. The filtrate is put into a separating funnel and is mixed with 20 ml of petroleum ether und 20 ml of 10% aqueous NaCl solution. The separating funnel is shaken carefully. When the layers have separated the lower layer is allowed to drain into a beaker. This phase is thrown away. The upper layer is washed 3-4 times with 5 ml of dest water. Afterwards the extract is placed in an Erlenmeyer flask and is dried with about 4 spatula tips of Na_2SO_4. The liquid is carefully decanted into a round bottom flask. Using a rotary evaporator the leaf extract is concentrated to a final volume of about 3 ml.

Application of the extract to the TLC plate:

With a pencil a line is drawn approximately 1.5 cm from the bottom of the plate. The coating of the plate should not be scraped! Using a paint brush or a Pasteur pipet the leaf extract is applied as a line to the TLC plate. The procedure is repeated until the line is very dark green. The transferred extract is allowed to dry thoroughly after each addition. The line is kept as thin and straight as possible.

Experimental procedure:

The loaded TLC plate is carefully placed in the TLC chamber with the sample line toward the bottom. The plate whose top is leaned against the jar wall should sit on the bottom of the chamber and be in contact with the developing solvent (solvent surface must be below the extract line). The TLC chamber is covered. When the solvent front has reached three quarters of the length of the plate, the plate is removed from the developing chamber and the position of the solvent front is immediately marked and calculate the Rf value

Discussion:

As the solvent rises by capillary action up through the TLC plate, the components of the pigment mixture are partitioned between the mobile phase (solvent) and the stationary phase (silica gel) due to their different adsorption and solubility strength. The more strongly a given component is adsorbed to the stationary phase, the less easily it is removed by mobile phase. The more weakly a component is adsorbed the faster it will migrate up the TLC plate. On the other hand, the running distance depends on the solubility of the pigment in the solvent. Since the experiment employs a high non-polar solvent (petroleum ether), the pigments that are least polar (carotenes) will be best solved in the non-polar solvent and will thus have the largest running distance.

Calculation:

$$\textbf{Rf value} = \frac{\text{Distance covered by solutes}}{\text{Distance covered by solvent}}$$

Observation:

Results

EXPERIMENT - 9

Determination of diacetyl content in dairy products

Introduction:

Diacetyl is an organic compound with the chemical formula $(CH_3CO)_2$. It is a yellow or green liquid with an intensely buttery flavor. Diacetyl occurs naturally in alcoholic beverages and is added to some foods to impart its buttery flavor.

Sour (cultured) cream, cultured buttermilk, and cultured butter are produced by inoculating pasteurized cream or milk with a lactic starter culture, churning (agitating) and holding the milk until a desired pH drop (or increase in acidity) is attained. Cultured cream, cultured butter, and cultured buttermilk owe their tart flavour to lactic acid bacteria and their buttery aroma and taste to diacetyl

Procedure of diacetyl measurement:

1. The extraction of diacetyl was performed using acetone. One millilitre of the diacetyl mixture sample was mixed with 1 ml of acetone and shaken vigorously for 30 s at 3000 rpm.
2. After centrifugation at 4000 g for 5 min the supernatant was removed for specific diacetyl quantification.
3. This fraction was kept at 80°C for less than 7 days. After filtration on a cellulose acetate filter (0.2 µm), the supernatant was directly injected into the gas chromatography apparatus.
4. Gas chromatography analysis of diacetyl was performed using a gas chromatograph equipped with a flame ionisation detector (GC-FID).
5. A capillary column (30 m × 0.32 mm, 0.25 µm film thickness) with a 100% polyethylene glycol phase was used.

Gas chromatography parameters were as follows:

- Helium carrier gas (1.5 ml/min), injected amount 5 µl, split mode injection at 1:10 splitting ratio, injector and detector temperatures 240 and 255°C, respectively;
- The oven temperature ran from 50°C to 240°C at 7°C/min with three isotherms: 2 min at 91°C, 3 min at 107°C, and 3 min at 186°C.
- The amount of detected molecules was expressed in surface area units (mV/min) calculated.

Observations :

Results:

Determination of total chlorophyll by Spectrophotometric method

Introduction:

Chlorophyll:

Chlorophyll is green pigments involved in photosynthesis in plant and some microorganism. Location of chlorophyll in plant cell: In plant cell, chlorophyll is present in chloroplast

Structure of chlorophyll:

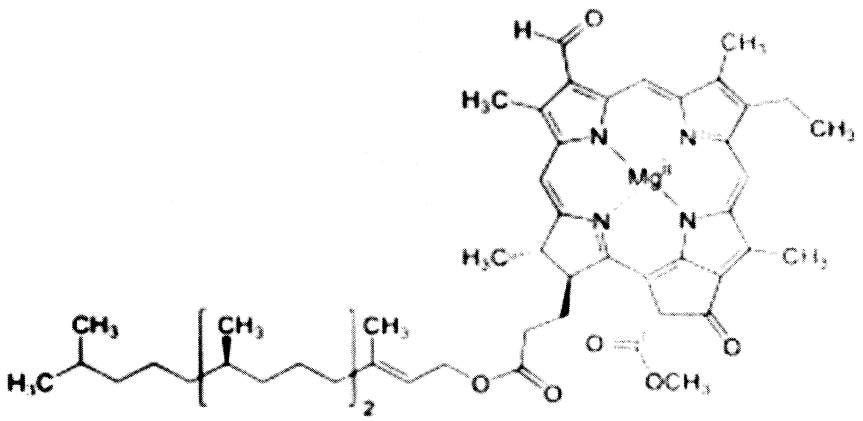


Fig. 1. Structure of Chlorophyll

Types of chlorophyll:

a. Chlorophyll-a
b. Chlorophyll-b

These are found in higher plants. They are present in the leaves in chloroplast normally in the ration 3:1

Preparation of spectrophotometer:

When a ray of monochromatic light of intensity 'lo' passes though a solution in a transparent vessel. Some of the light is observed. So that intensity of the transmitted light 'l' is less than 'lo'. There is some loss of light intensity from scattering by partials in the solution and refection but mainly form absorption by the solution.

1. **Lambert law:** Amount of light absorbed in directly proportional to thickness of solution being analyzed.

2. **Beer's law:** Amount of light absorbed in directly proportional to concentration of solution

Requirement: Food sample, mortal and pistol, filter paper, centrifuge, spectrophotometer.

Procedure:

1. Take 1g of sample
2. Finely chalked and grind with the help of 80% acetone
3. Filter the content
4. Repeat procedure till the pallet become colourless
5. Centrifuge the content at 5000 rpm for 6 min
6. Finally make volume to 80% acetone to 10 ml
7. Take 80% acetone as a blank

Observation: Record optical density at 645nm-663nm

Calculation: Calculate a and b or total using following formulas

c. Chlorophyll-a

$$= \frac{w\,[12.7 \times \text{O.D. at } 663\text{nm}] - [2.69 \times \text{O.D. at } 645] \times V}{1000}$$

d. Chlorophyll-b

$$= \frac{w\,[22.9 \times \text{O.D. at } 645\text{nm}] - [4.48 \times \text{O.D. at } 663] \times V}{1000}$$

Where,

W= Wt. of sample

V= Volume of extract (1000ml)

Results :

Detection of Chemical Preservatives in Foods

Qualitative test for detection of Propionic Acid and Acetic Acid

Principle:

Acids such as acetic, propionic, butyric and Valeric acids are steam distilled and the distillate is concentrated after neutralization. Separation of these acids is achieved by Paper Chromatography and visualized by spraying with methyl red and bromothymol blue.

Reagents:

1. Developing solvent- Acetone: tertiary-butanol: n-butanol: ammonia (2+1+1+1) Prepare fresh.
2. Spray reagents: Add 200 mgs of each of methyl red and bromothymol blue to a mixture of 100 ml of formalin and 400 ml of alcohol. Adjust pH to 5.2 with 0.1N sodium hydroxide.

Standard acid solution:

Pipette 1 ml each of acetic, propionic, butyric and valeric acids into 100 ml volumetric flasks separately and dilute to volume with water.

Pipette 1 ml of each stock solution into 25 ml beakers and 1 ml each into another beaker (mixture), neutralise with 0.1 N sodium hydroxide using cresol red indicator and evaporate to dryness without charring. Dissolve in 0.5 ml water. Use these solutions for chromatography.

Procedure:

1. Steam-distill 20 gm of well mixed sample and collect 200 ml distillate.
2. Immediately neutralise the distillate with 0.1N sodium hydroxide using cresol red indicator and evaporate just to dryness and dissolve in 0.5 ml water.
3. Spot 1-2 µl along with standards on Whatman No.1 paper and allow them to air dry. Clip paper to glass rod and suspend in a tank with 50 ml mobile phase in a trough (since mobile phase is heavy, use 3 clips to hold the paper to glass rod to prevent sagging).
4. Develop the chromatogram approximately to 2.5 cm from top of the paper, remove and let air dry. Spray with the spray reagent (spray should be uniform).
5. Faint yellow spots indicate presence of acids, heavier blue spots are due to sodium ion. To intensify spots, expose paper to ammonia fumes. Entire paper immediately turns to green and acids gradually appear as red spots. Since colour of acids is not stable, mark spot with pencil as soon as they are completely developed.
6. Identify them in sample from the Rf values of standard acids.

2. Qualitative test for Sorbic acid:

UV Spectrophotometric Method: (Applicable to fresh dairy products, cottage cheese, ricotta and mozzarella cheese, sour cream and yoghurt)

Principle:

Sorbic acid is extracted from the sample using the solvent mixture of diethyl ether and petroleum ether (1: 1) and absorbance of the extract is measured at 250 nm. Sorbic acid in another aliquot is destroyed with permanganate and absence of the peak at 250 nm is taken as confirmation of the presence of sorbic acid in the sample.

Apparatus: Spectrophotometer

Reagents:

1. Metaphosphoric acid Solution: Dissolve 5 gm in 250 ml water and dilute to 1 L with alcohol.
2. Mixed ethers (40-60): Petroleum ether and anhydrous diethyl ether (1+1).
3. Potassium permanganate solution: Dissolve 15 gm in 100 ml water.
4. Sorbic acid standard solution (1 mg/ml): Dissolve 100 mg and make up to 100 ml with mixed ethers.
5. Woking standard solution: Dilute 5 ml to 100 ml with the solvent.
6. Reference solution: Shake 10 ml of mixed ethers with 100 ml of phosphoric acid solution and dry the supernatant ether layer with anhydrous sodium sulphate.

3. Procedure:

1. Homogenize the food sample by cutting into small pieces using a food chopper or by shredding it over a sieve. With creamed Cottage and similar cheeses place 300 – 600 gm of sample at 15°C in a 1 L cup of a high speed blender and blend for the minimum time (2 – 5 min) required to obtain a homogeneous mixture.
2. Accurately weigh about 10 gm of the prepared sample, in a high speed blender, add enough phosphoric acid to yield a total of 100 ml of liquid in the mixture.
3. Blend for one minute and immediately filter through Whatman No.3 paper. Transfer 10 ml of filtrate to a 250 ml separator containing 100 ml of mixed ethers and shake for one minute.
4. Discard the aqueous layer and dry the ether extract over 5 gm of anhydrous sodium sulphate and read the absorbance at 250 nm against reference solution.
5. Determine the concentration of sorbic acid from the standard curve prepared as follows
6. Add 1, 2, 4 and 6 ml of working standard solution to 100 ml volumetric flask and dilute to volume with mixed ethers.
7. Determine the absorbance at 250 nm against mixed ethers Plot absorbance (A) against mg sorbic acid / 100ml determine the sorbic acid content of the sample from its absorbance by making use of standard curve.
8. The final result may be expressed in ppm.

$$\textbf{\% Sorbic acid} = (\text{mg of sorbic acid / gm sample}) \times (1 / 1000) \times 100$$

Observations:

Results:

EXPERIMENT - 12

Role of Acidulants in Food

Introduction:

Acidulants are chemical compounds that confer a tart, sour, or acidic flavour to foods. Acidulants are additives that give a sharp taste to foods. They also assist in the setting of gels and to act as preservatives. The pH of a food is a measure of its acidity, alkalinity or neutrality. Acidulants are acids used in processed foods for a variety of functions that enhance the quality of food in many other ways as:

Flavoring agent: Contributes and enhances flavor in carbonated beverages, fruit drinks, and desserts.

Preservative: An acid medium restricts the growth of spoilage organisms in mayonnaise and tomato sauce, and retards the activity of enzymes involved in discoloration in fruits.

Chelating agent: Aids in binding metals that can cause oxidation in fats and oils, and discoloration in canned shrimp.

Buffer: Maintains and controls acidity during processing, and maintains acidity within a given range in prepared desserts.

Gelling agent: Controls the gelling mechanism of algin and pectin gels such as desserts and jams.

Coagulating agent: Reduction of pH results in coagulation of milk protein which is used in the preparation of direct acidified cheese and desserts.

Many natural foods are acidic. For example, oranges, lemons, apples, tomatoes, cheese and yoghurt contain natural acids, such as citric acid, that give them their characteristically sharp taste.

As the food industry has developed, so has the growth in production of processed foods. Many of these need the inclusion of an acidulants to give an acidic or sour taste.

Applications of acids in Food

By far the most important, versatile and widely used organic acid is citric acid. It is used in food products, drinks and the pharmaceutical industry. The list below shows some common used acidulants:

1. **Acetic acid**

- This is the acid found in vinegar and has a characteristic pungent smell.
- Acetic acid is widely used, particularly in the pickling industry. Naturally fermented vinegar has a variable pH and so acetic acid is added to this to form pickling liquor with a specified acidity.
- It can also be used in confectionery goods and flavourings. The flavouring sodium diacetate is commonly known as 'salt 'n' vinegar' and is widely used in crisps. Acetic acid has excellent bacteriostatic properties and hence has considerable importance as a preservative.
- Only acetic acid produced naturally by fermentation can legally be called vinegar. In Britain the main carbohydrate used is usually malt and so the vinegar it produces is called Malt Vinegar. Acetic acid can also be manufactured synthetically by various methods

2. Citric acid

Citric acid is widely used in the food industry to:

- Provide sharp taste in soft drinks and sweets
- Generate the optimum conditions for the formation of gels in jams, jellies, confectionary and desserts
- Help give the conditions for the stabilisation of emulsions (e.g. processed cheese and dairy products)
- Prevent the browning of salads
- Enhance the action of antioxidants and prevent deterioration in frozen food
- Act as an antioxidant in fats and oils
- Preserve meat products and help modify their texture during their processing

Citric acid was originally extracted from lemons and limes but it is now produced commercially by a fermentation process. The mould Aspergillus niger is used to ferment a carbohydrate source such as molasses

3. Fumaric acid

- Fumaric acid is the strongest tasting food acidulant. It has limited applications due to its very low solubility. In the main, it is used in gelatin dessert powders, cheesecake mixes and some powdered drinks.
- A substantial amount of fumaric acid is used in animal feedstuffs mainly because of its strong flavour and favourable price.
- It is manufactured synthetically from malic acid.

4. Lactic acid

- Lactic acid is widely used in the production of boiled sweets, pickled foods and as a raw material in the manufacture of important emulsifiers for the the baking industry.
- It contributes to smell, taste, texture and colour of the food. Lactic acid also used to enhance broad range of savory flavours.
- Because of its mild taste, lactic acid is used as an acidity regulator in beverages such as fruit juices and soft drinks.
- It is produced during anaerobic respiration and is commonly manufactured by a fermentation process, although it can be produced synthetically.

5. Malic acid

- Malic acid is found naturally in apples, pears, tomatoes, bananas and cherries. It has similar applications to citric acid and is the preferred acid in low calorie drinks, cider and apple drinks.
- However, it has the disadvantage of being slightly more expensive than citric acid.
- Malic acid is a naturally occurring substance found in many fruits and vegetables, and largely responsible for sour taste in apples and pears.
- Malic acid is used as a flavour enhancer in a variety of foods including hard and soft candies, water ices, chewing gum, fruit preserves and bakery items.
- It also acts as a preservative to in carbonated and non-carbonated beverages.
- It is produced commercially from maleic anhydride.

6. Phosphoric acid

- Is the acidulant used in the second largest amounts by the food industry? This is because of its use in one single product that is produced in massive amounts: cola drinks.
- Cola drinks are the best selling flavoured soft drink in the world. The acid used in these drinks is exclusively phosphoric acid. This has a harsh, biting taste which complements the cola flavour.
- Salts of phosphoric acid have many uses in the food industry. They can act as buffers, acidulants for baking powders and emulsifying salts in the production of processed cheese.
- Phosphoric acid is manufactured commercially from phosphate rock mined principally in North Africa and North America.

7. Tartaric acid

- This was the first food acidulant to be used in significant quantities although its use has now been mostly replaced by citric acid. The largest single application for tartaric acid is as a raw material for the manufacture of the emulsifier's bread improvers.
- Tartaric acid can be manufactured by natural and synthetic routes. The natural route involves the recovery of tartaric acid from wine. The synthetic route involves the chemical reactions of maleic anhydride.
- An important salt of tartaric acid, potassium hydrogen tartrate (or cream of tartar), has applications as an acidulant for baking powder and sugar confectionery.

EXPERIMENT - 13

Study of effect of stabilizers/thickeners on quality of foods

Hydrocolloids are defined as 'a macromolecular substance such as a protein or polysaccharide which swells by absorption of water, in some cases forming a stiff gel. Food gums are usually added to food systems/products for specific purposes, such as thickening agents, stabilization, gelling, etc. Hydrocolloids ultimately alter the rheological properties in a desired fashion for food systems.

Ideal characteristics of a food grade Stabilizer / Thickeners.

They should be nontoxic
They should readily disperse in the mix
They should not produce excessive viscosity or separation or foam in the mix
They should not clog strainers and filters
They should not impart off flavor to the product
They should be economical

Types of Stabilizers / Thickeners.

There are a variety of hydrocolloids on the market, including those derived from plants or seaweed, and those produced by microorganisms. In general, hydrocolloids have a sugar backbone that contains protruding substituents such as esters, sulfates, or additional sugars. Hydrocolloids available for food applications are either neutral or negatively charged.

A. Gelatin:

Gelatin is a proteinaceous material obtained from animal connective tissue (collagen) using hydrolysis in acidic (type A) or basic (type B) solution followed by hot water extraction. Commercially, skins or bones of different animal species, such as beef, pork, fish and poultry, form the main raw material for gelatin production. It hydrates readily in warm or hot water to give low-viscosity solutions that have good whipping and foaming properties. After cooling, the network of polypeptide chains associates slowly to form clear, elastic gels that are syneresis free. This relatively expensive stabilizer is effective at concentrations of 0.3–0.5%; however, it may not prevent the effects of heat shock. It is also not acceptable to certain religious and vegetarian populations. The use of gelatin as a stabilizer produces thin mixes that require a long aging period. Gelatin disperses easily and does not cause wheying-off or foaming.

B. Galactomannans:

Guar gum and locust bean gum (LBG), both classified as galactomannans with the same mannose backbone, are used in products such as cheeses, frozen desserts, processed meats, and bakery products. However, these two galactomannans differ in cold water solubility as well as in their gelling capability due to their difference in the degree of substitution and the distribution of side units.

C. Guar gum:

Guar gum is extracted from the seeds of a tropical legume, Cyamoposistetragonolba, which is milled in order to obtain guar gum. Guar gum is a neutral hydrocolloid with linear chains of D-mannopyranosyl units with D-galactopyranose substituents protruding by (1→6) linkages. For every galactose residue there are approximately two mannose residues. Guar gum is highly substituted which allows for good hydration and hydrogen bonding activity. The molecular weight (MW) of guar gum is between 220,000 and 300,000. Guar gum has a higher degree of galactose substitution (40%) than locust bean gum (20-23%). On an average, for every two molecules of mannose, a galactose side unit is attached. Guar gum is stable over a wide range of pH, with its optimal rate of hydration between pH 7.5-9.0.

D. Carrageenan (Irish moss):

The red seaweed family, Rhodophycae, provides the polysaccharides agar, carrageenan, and furcellaran. Individual plants that grow together will produce both types of carrageenan. Carrageenan gum, a negatively charged hydrocolloid has a linear backbone of repeating galactose units with different proportions and locations of ester sulfate groups and 3,6- anhydrogalactose (anhydro bridges).

E. Gum Arabic:

Gum Arabic (Acacia gum) is a natural, vegetable exudate from acacia trees (primarily in Africa). The main chain of this polysaccharide is built from (1→ 3) and (1→ 6) linked β-Dgalactopyranosyl units along with (1→ 6) linked β-D-glucopyranosyluronic acid units. Side branches may contain β-L-Rhamnopyranose, β-D- Glucuronicacid, β-D-Galactopyranose, and β-L-Arabinofuranosylunits with (1→3), (1→4), and (1→6) glycosidic linkages. Gum arabic has a high water solubility (up to 50% w/v) and relatively low viscosity compared to other exudate gums.

F. Xanthan:

This bacterial exopolysaccharide is obtained by the growth of Xanthomonascampestris in culture. Xanthan gum is produced by the process of submerged aerobic fermentation using glucose as the primary carbohydrate source. The xanthan gum is recovered, purified, dried and milled into a white powder. Xanthan gum is an anionic (negatively charged) linear hydrocolloid with a (1→4) linked β-D-glucose backbone. The side unit, a trisaccharide, contains a glucuronic acid residue linked (1→4) to a terminal mannose unit and (1→2) to a second mannose which connects to the glucose backbone.

Generally, xanthan gum is stable over the pH range 2 to 12. The functionality of xanthan gum is highly dependent on the ionic strength of the solution.

It is an excellent thickening agent. It exhibits pseudoplastic rheological characteristics (i.e. as shear is increased, viscosity gets reduced). Its blend with guar gum and/or LBG, it makes an effective stabilizer for ice cream, ice milk, sherbet, and water ices. A combination of xanthan gum with sodium alginate is reported to serve as a milk shake stabilizer.

G. Alginates:

Alginates constitute the primary structural polysaccharides of brown seaweeds (Phaeophyceae). Alginates, or algin, are a generic term for the salts and derivatives of alginic acid (E400). Alginates are unbranched copolymers of (1→4)-linked □-D-mannuronic acid (M) and □-Lguluronic acid (G) residues. If the uronic acid groups are in the acid form (–COOH), the polysaccharide, called alginic acid, is water insoluble. The sodium salts of alginic acid (-- COONa), sodium alginates (E401), are water soluble. Alginates dissolve in cold water and gel in the presence of calcium and acid.

H. Gellan gum:

Gellan gum is a fermentation polysaccharide produced by the microorganism Sphingomonas elodea. The molecular structure of gellan gum is a straight chain based on repeating glucose, rhamnose and glucuronic acid units. Upon cooling of gellan solutions, the polysaccharide chains can assume double helices, which aggregate into weak gel structures. In the presence of appropriate cations (Na+ or Ca++), the double helices form cation-mediated aggregates, which leads to formation of strong gel networks.

Study of effect of clarifying agents on the fruit juices

Introduction :

Clarifying agents are used to remove suspended solids from liquids by inducing flocculation the solids begin to aggregate forming flakes, which either precipitate to the bottom or float to the surface of the liquid, and then they can be removed or collected.

It works on the principle that all of the particles responsible for the clouding or haze in a wine or beer have an electrical charge. As an example gelatin has a positive charge meaning that it can attract negatively charged materials. In binding to the negatively charged materials the combined weight increases resulting in settling to occur. In practice it's usually necessary to have clarifying agents of different charges added sequentially to the wine in order remove the materials of various charges contained in the wine.

Clarifying agents:

Bentonite, Alum, Gelatin, Aluminium Chlorohydrate, Gelatin, Aluminium sulphate, Calcium Oxide, Calcium hydroxide, Ferrous sulphate, Ferric chloride, Polyacry lamide, Sodium aluminate and Sodium silicate. etc

Bentonite :

Bentonite negative charge attracts positively charged particles, such as protein, to its surface and gradually carries them down due to the forces of gravity. Bentonite absorbs a large quantity of water, which increases its surface area and aids in deproteinizing. The alkaline/basic nature of bentonite results in a rapid reaction in the acidic wine resulting in simultaneous combination with proteins & other positively charged particles. Bentonite is relatively non-specific, absorbing all proteins. It has good flavour retention property, protein stability and Doesn't affect tannin levels.

Gelatin :

Gelatin is colloidal in nature and primarily has a positive charge. Gelatin requires tannin to flocculate which limits its to use primarily red wines. It attracts tannins which are primarily negatively charged. Once this neutralization has occurred the turbid particles tend to agglomerate which in turn causes them to settle out. Acts on both proteins and tannins. Gelatin can also be used to preserve clarity, and improve the sensory qualities, soften the wine and balance the composition.

EXPERIMENT - 15

Role of Emulsifiers in Foods

General Introduction:

Emulsifiers:

A food additive, which forms or maintains a uniform emulsion of two or more phases in a food. Emulsifiers allow water and oils to remain mixed together in an emulsion, as in mayonnaise, ice cream, and homogenized milk. It stops fats from clotting together.

Types of emulsifiers:

i. Clouding agent
ii. Crystallization inhibitor
iii. Density adjustment agent (flavouring oils in beverages)
iv. Dispersing agent
v. Emulsifier
vi. Plasticizer
vii. Surface active agent
viii. Suspension agent

1. Emulsifier in Bakery products:

Emulsifiers can function as dough conditioners:

Advantages attributed to dough conditioners include

i) improved tolerance to variations in flour and other ingredient quality,
ii) doughs with greater resistance to mixing and mechanical abuse,
iii) better gas retention resulting in lower yeast requirements, shorter proof times, and increased baked product volume,
iv) increased uniformity in cell size, a finer grain, and a more resilient texture,
v) stronger sidewalls,
vi) reduced shortening requirements, and
vii) improved slicing

- Certain emulsifiers also function as crumb softeners, another type of dough conditioner. The soft crumb, a characteristic of freshly baked bread, can be retained longer if the appropriate emulsifiers are added.
- Crumb firming, associated with staling and starch retrogradation, can be delayed for 2–4 days with the addition of emulsifiers.
- Emulsifiers reduces the rate of water migration and hence, staling, by complexing with the starch and adsorbing onto the starch surface.

- Cake volume can be increased upon addition of several different emulsifiers, including diacetyl tartaric acid esters of monoglycerides, glycerol fatty acid esters, and calcium stearoyl-2-lactylate.
- Emulsifier addition to cake mixes facilitates increased substitution of sucrose with high-fructose corn syrup. Fructose causes starch gelatinization earlier during baking, relative to sucrose, reducing cake volume, grain size, textural quality, and shelf-life.
- Emulsifiers increase the gelatinization temperature offsetting the effect of the high-fructose corn syrup. Emulsifiers have three primary functions in cake:

i) to facilitate air incorporation,
ii) to disperse shortening in smaller particles to allow the maximum number of air cells, and
iii) to improve moisture retention.

- Cookie characteristics (volume, top grain, and, particularly, spread ratio) can be improved with the addition of the appropriate emulsifier.
- Cookie shelf-life can also be increased with addition of emulsifiers. Emulsifier addition to cookie mix can improve the spread ratio, cooking surface release, and texture of cookies.

2. Dairy Products:

- Emulsifiers in ice cream improve fat dispersion, facilitate fat–protein interactions, control fat agglomeration, facilitate air incorporation, impart dryness to formed products, confer smoother texture due to smaller ice crystals and air cells, increase resistance to shrinkage, reduce whipping time, and improve melt-down.
- These emulsifiers were found to increase stability to heat shock and improved the body and texture of low fat ice cream.
- The monoglycerides improve foamability, impart solidity, and improve the shape-retaining characteristics of the frozen product. The improved foamability is due to interaction of the emulsifier with milk proteins.

3. Candy Products:

- The elimination of "bloom," i.e., the transition of fat crystals from the alpha and beta' configuration to the less desirable beta configuration, is a key reason for the addition of emulsifiers to candy products.
- Emulsifiers can be used as crystal structure modifiers in mixtures of triglycerides. Certain emulsifiers can also be used to control product viscosity in cream fillings and in chocolates.
- Emulsifiers can be effective in preventing chocolate bloom. Emulsifiers are often used with both semisweet and milk chocolate.
- Emulsifiers aid in processing by reducing the weep or exudate that occurs with heavy sugar pastes during processing.

4. Miscellaneous:

- Emulsifiers have been utilized in the production of meat analog products. Emulsifiers are used in the formulation of flavor emulsions, although the available literature is rather limited.
- Emulsifiers fulfil three functions in margarine:

i) assistance in emulsion formation,

ii) modification of crystal structure in the vegetable fat, and
iii) antispattering. Typically, a mixture of lecithin or citric acid monoglycerides and monoglycerides are used. To reduce sandiness and "oiling out" in margarine due to recrystallization.

- Lecithin, contained in the added egg yolk, is usually the only emulsifier added. Emulsifier in vegetable oil added to inhibit crystallization, e.g., ethoxylated sorbitan monooleate and monostearate.
- To inhibit cloud formation in salad dressings and salad oils, emulsifiers can also be added.
- To improve wetting characteristics in instant cocoa drinks, the powder is agglomerated. Lecithin will facilitate agglomeration during spraydrying.
- Emulsifiers are often used in peanut butter. The addition of an emulsifier to peanut butter can inhibit oil phase separation.

Role of Leaving Agents in Bakery Food Product

General Introduction:

The leavening agents are substances used in doughs and batters for leavening. These agents produce a gas which expands during baking, leave small holes in the baked food product and lightens and softens the finished product. The gas produced may be air incorporated by mechanical means but usually it is carbon dioxide produced by biological agents, or by chemical agents reacting with moisture, heat, acidity, or other triggers. When baked, the porosity translates into the crumb of the finished product.

Leavening agents, thus, contribute significantly to the textural properties of baked products by expanding the batter or dough, sometimes during mixing and always during baking. These also tenderizes the crumb and contributes to the aesthetic enjoyment of the final product by giving it uniform cell structure, bright crumb color, soft texture and enhanced palatability.

There are three types of leavening agents:

1. Mechanical leavening agents
2. Chemical leavening agents
3. Biological leavening agents

1. Mechanical leavening agents:

Leavening can be achieved mechanically by integrating tiny air bubbles into the mixture using a mixer or whisk. Creaming, a process employed in cookie and biscuit production is such a process in which incorporation of air bubbles is achieved by beating fat and sugar crystals together in a mixer. Creamed mixtures are usually further leavened by a chemical leavener like baking soda. Beating of certain liquids like egg white or cream using a whisk is another method of air incorporation employed in cake manufacture. The air incorporated expands during heating resulting into raising in the baked products.

2 Chemical leavening agents:

The chemical leavening agents are chemical mixtures or compounds that release gases (usually carbon dioxide) when they react with moisture and heat. The gas produced is held by fat pockets, gluten and starch, which makes the baked product rise. Chemical leavening is used in quick breads, cakes, cookies etc. where a long biological fermentation is impractical or undesirable.

There are various types of chemical leavening agents but some of the most frequently used include baking soda, baking powder, sodium bicarbonate, potassium bicarbonate, hydrogen peroxide, etc. The amount of leavening agents in formulation has great effect on the final baked product quality. Too much leavening agent will make the bubbles too big, resulting into combination and bursting of these bubbles, leading to a flat cake or bread. Too little leavening agent will result in a heavy product, with soggy or damp layers.

Baking powder:

Baking powder is a mixture of baking soda, dry acids (usually low molecular weight organic acid) and a filler, usually corn starch or calcium carbonate. The acid salt prevents the production of sodium carbonate which causes bitterness, unpleasant, soapy flavour as well as a yellowish colour in the product. The filler serves following purposes:

1. To keep the baking powder free flowing
2. To provide bulk
3. To keep the baking soda and the acid dry so that no reaction occurs while in storage.
4. To help in releasing the standard amount of CO_2.

Sometimes powdered and dried egg albumen is also added to baking powder as filler. Egg albumen powder increases the viscosity of the dough which helps to hold gas bubbles in the dough. Hence the effectiveness of baking powder is increased.

Baking powder is a very widely used ingredient in cooking and baking such things as buns, fruit loaves, crumpets, pikelets, pastries, cakes, pies, biscuits, omelettes, some savouries and some puddings. Baking powder when used in the formulation releases CO_2 during baking according to the following equation:

$$NaHCO_3 + H+ \text{ (from the acid)} \rightarrow Na+ + H_2O + CO_2$$

Using cream of tartar ($KHC_4H_4O_6$) also known as potassium bitartrate in a recipe along with baking soda, will produce CO_2 by a two-step reaction. In the first step, the cream of tartar and the baking soda react, producing sodium potassium tartrate and carbonic acid:

$$NaHCO_3 + KHC_4H_4O_6 \rightarrow KNaC_4H_4O_6 + H_2CO_3$$

In the second step, the carbonic acid is broken down into carbon dioxide and water as follows:

$$H_2CO_3 \rightarrow H_2O + CO_2$$

The time and rate of gas evolution from baking powder can be regulated by the selection of different baking acids that react faster or slower with sodium bicarbonate. There are three different kinds of baking powder - fast acting, slow acting and double acting which differ in their gas (CO_2) releasing action

Ammonium carbonate or bicarbonate:

Ammonium carbonate or bicarbonate is used as leavening agent in biscuits and crackers etc. that are to be baked at low moisture. Upon heating it produces CO_2 and NH_3 gases and no solid is left behind in this reaction. However if it is not removed completely it may impart detectable odours. Hence its use is limited to the biscuits and crackers which have large surface to mass ratio and ammonia will be escaped easily at high temperatures

Biological leavening agents:

The leavening in bread is achieved by means of baker yeast (*Saccharomyces cerevisiae*) or a portion of fermenting sponge which consist of living microbes. Yeast is an oval shaped, single celled and colourless fungus that produces carbon dioxide gas through fermentation. It is active in air as well as in absence of air. It grows rapidly in presence of air but produces more alcohol in absence of air. Yeast is universally distributed, generally growing harmlessly on various plant parts wherever sugar is available. During fermentation, the yeast converts glucose into ethyl alcohol and carbon dioxide as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Glucose Ethyl alcohol + carbon dioxide

During baking the ethyl alcohol gets evaporated while the carbon dioxide gas entrapped in the gluten film causes the product to rise. The optimal temperature for yeast fermentation is27°C (80°F). Baking stops the fermentation process as the high heat kills the yeast. The functions of yeast in bread making are as follows-

1. Fermentation of the dough.
2. To lighten the dough.
3. To impart characteristic aroma and flavor to the bread.

Other leavening agents Steam:

Water present in the batter also acts as leavening agent when it expands upon heating. To take advantage of this style of leavening, the baking must be done at high enough temperatures to flash the water to steam, with a batter that is capable of holding the steam in until set. Cream puffs, popovers and pie crusts are steam leavened products.

EXPERIMENT - 17

Role and Mode of Action of Antioxidants in Food Products

Introduction:

- Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. The applications of antioxidants have been widespread in the food industry for preventing lipids from oxidative degradation.
- Fats, oils and lipid-based foods deteriorate through several degradation reactions both on heating and on long term storage. The main deterioration processes are oxidation reactions and the decomposition of oxidation products which result in decreased nutritional value and sensory quality.
- The main proposed mechanisms through which the antioxidants may play their protective role, including free radicals inactivating, the hydrogen atom transfer, pro-oxidative metals chelating, the single electron transfer, quenching of singlet oxygen as well as photo-sensitizers and lipoxygenase inactivation.

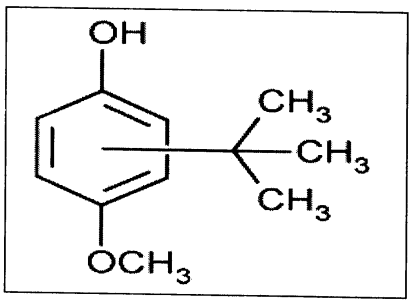
Types of Antioxidants

- **Natural antioxidants:**

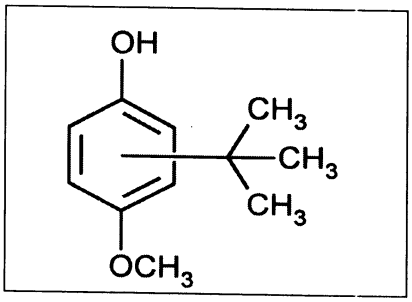
1. Tocopherols (delta>gamma>beta>alpha)
2. Nordihydroguaretic Acid (NDGA)
3. Sesamol
4. Gossypol

- **Synthetic antioxidants:**

1. Butylated Hydroxy Anisole (BHA)
2. Butylated Hydroxy Toluene (BHT)
3. Propyl Gallate (PG)
4. Tertiary Butyl Hydroquinone (TBHQ)



Butylated Hydroxy Anisole (BHA)



Butylated Hydroxy Toluene (BHT)

Mechanism of autoxidation

As a free-radical reaction, autoxidation proceeds in three distinct steps:

1. **Initiation:**

- The first step is initiation in which lipid radicals are formed from lipid molecules. Abstraction of a hydrogen atom by a reactive species such as a hydroxyl radical may lead to initiation of lipid oxidation.
- Secondary initiation by homolytic cleavage of hydroperoxides is a relatively low energy reaction, and it is normally the main initiation reaction in edible oils. This reaction is commonly catalysed by metal ions.

2. **Propagation**

- After initiation propagation reactions occur in which one lipid radical is converted into a different lipid radical. These reactions commonly involve abstraction of a hydrogen atom from a lipid molecule or addition of oxygen to an alkyl radical.

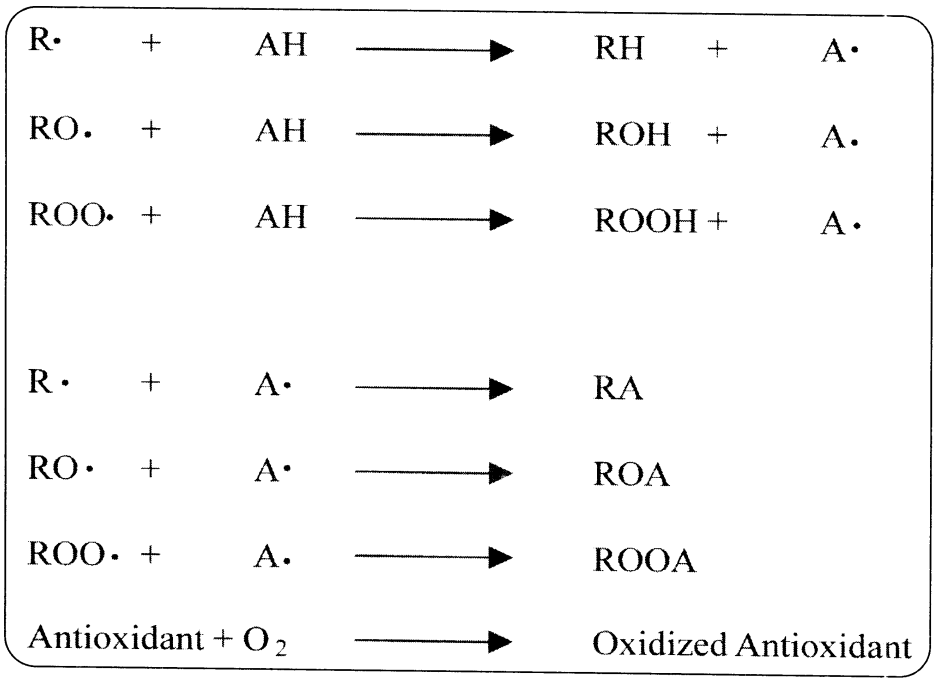
Initiation	$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$
Propagation	$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$
	$ROO^{\bullet} + R'H \longrightarrow ROOH + R^{\bullet}$
Termination	$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$
	$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$
	$R^{\bullet} + R^{\bullet} \longrightarrow RR$
Secondary initiation	$ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$
	$2\,ROOH \longrightarrow RO^{\bullet} + ROO^{\bullet} + H_2O$
Metal-catalysed initiation:	
	$M^{n+} + ROOH \longrightarrow RO^{\bullet} + {}^{-}OH + M^{(n+1)+}$
	$M^{(n+1)+} + ROOH \longrightarrow ROO^{\bullet} + H^{+} + M^{(n)+}$

- The enthalpy of reaction is relatively low compared with that of the initiation reactions, so propagation reactions occur rapidly compared with initiation reactions. At normal atmospheric pressure of oxygen, the reaction of alkyl radicals with oxygen is very rapid, and the peroxy radicals are present at much higher concentrations than the alkyl radicals.
- Abstraction of hydrogen takes place preferentially at carbon atoms where the bond dissociation energy is low. Since the bond dissociation energy of the C–H bond is reduced by neighbouring alkene functionality, abstraction of hydrogen takes place most rapidly at the methylene group between two alkene groups in a polyunsaturated fatty acid (PUFA).

3. Termination:

- Termination reactions in which free radicals combine to form molecules with a full complement of electrons are low energy reactions but are limited by the low concentration of radicals and by the requirement for radicals with the correct orientation for reaction to collide. However, in frying oils termination reactions are important, with dimers and higher polymers contributing to the increased viscosity of the oil.

- **Reaction with free radicals**



Mechanism of Action of antioxidants:

- Antioxidants slow down the oxidation rates of foods by a combination of scavenging free radicals, chelating pro-oxidative metals, quenching singlet oxygen and photo-sensitizers, and inactivating lipoxygenase.

Free radical scavenging

- Examples of antioxidants to scavenge free radicals are phenolic compounds (tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), lignans, flavonoids, and phenolic acids), ubiquinone (coenzyme Q), carotenoids, ascorbic acids, and amino acids. Thiacremonone extracted from heated garlic at 13^{0}C has higher radical scavenging activity than ascorbic acid, a-tocopherol, or BHA.

- The effectiveness of antioxidants to scavenge free radicals in foods depends on the bond dissociation energy between oxygen and a phenolic hydrogen, and reduction potential and delocalization of the antioxidant radicals.
- Vitamin E or a-tocopherol is a methylated phenol required in the human diet. Phenolic compounds primarily inhibit lipid oxidation through their ability to scavenge free radicals and convert the resulting phenolic radicals into a low-energy form that does not further promote oxidation.
- The tocopherol radical sometimes reacts with lipid peroxy radicals at very high concentration and produces tocopherol peroxide. Tocopherol peroxide produces two isomers of epoxy-8 -hydroperoxytocopherone which becomes epoxyquinones upon hydrolysis.
- This reaction produces alkoxy radicals, instead of peroxy radicals, and loses only tocopherol. Since there is no net decrease in free radicals in the system, tocopherol does not act as an antioxidant.