UNIT 5 TESTING AND EVALUATION: CHEMICAL AND MICROBIOLOGICAL

Structure

5.0 OBJECTIVES

After reading this unit, you should be able to:

- describe the methods for determining crude fat, protein, pectin and tannins in food products;
- discuss various aspects of bacteriological examination of water;
- describe procedures for assessing surface sanitation; and
- explain the salient aspects of microbiological examination of spoiled canned foods.

5.1 INTRODUCTION

Analytical food chemistry deals with the methods for determining the chemical composition (quality) of foods. It employs both qualitative and quantitative methods for the purpose. A qualitative method yields information about the nature of the component and a quantitative method provides numerical information on the content of the component in the sample.

For the analysis of foods, chemical methods are more often employed even though physical methods are also proving to be very useful. The analytical methods based on the physical properties of food components are collectively known as “Instrumental Methods of Analysis’, which you will be learning in the next Block.

You will be learning and performing several analytical methods in your Practical exercises under Courses III and VII. In this unit, you will learn a few other methods, which are, either too time consuming or could not be included under the Practical exercises because they require more sophisticated facilities which are available only in specialised laboratories.

Microbiological quality of foods is equally important. A major portion of microbiological methods will be covered under Course V 'Food Microbiology". Therefore, in this Unit, only a selected few methods are discussed.

5.2 CHEMICAL ANALYSIS OF FOODS

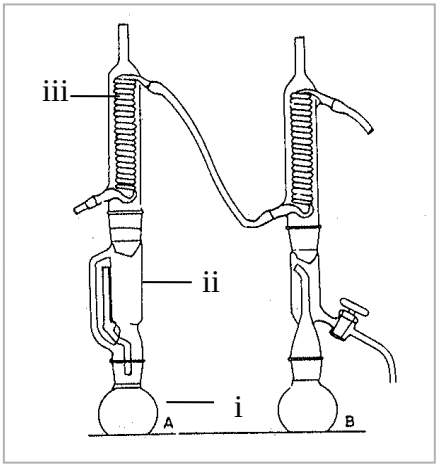
The quantitative analysis of food raw materials and their products may be classified into **proximate analysis** and **ultimate analysis**. Proximate analysis provides information on the nutritional and biochemical composition, while ultimate analysis or detailed analysis determines the content of a particular component in the food material.

The proximate analysis, especially for fruits and vegetables consists in determining the percentages of the moisture, ash, acidity, crude fat or ether extractives, protein, sugars and crude fibre. Their sum total subtracted from 100 represents primarily the amount of carbohydrates other than sugars, but includes starch, pectin, etc.

Among the above constituents, all except **crude fat, protein** and **pectin** are covered in the Practical exercises**.** Therefore, these methods are described in this unit. Tannin, which is another component of importance to fruits and vegetables, is also included here.

5.2.1 Crude Fat or Ether Extractives

As you have learnt already, food products contain water-soluble and water insoluble constituents. Among the water insoluble constituents, the ether soluble materials such as the tri- glycerides, phospholipids, sterols, essential oils, fat-soluble vitamins and pigments are very important. The first step in determining most of the individual components is extracting the food material with ether using a Soxhlet extraction apparatus (Figure 5.1). The Soxhlet extraction apparatus is an all glass simple assembly consisting of a Soxhlet flask to the top of which a Soxhlet extraction tube and a condenser are attached through standard glass joints. The Soxhlet extraction tube has provision for siphoning off the solvent used for the extraction when the level has reached a particular level.



i. Soxhelt flask
ii. Soxhelt extraction tube
iii. Condensor

Figure 5.1: Soxhlet apparatus (A) for fat extraction and (B) for solvent removal

Ether extractives are determined on samples after moisture determination (please see 'Moisture determination' under Course III Practical exercises). The dried sample is transferred to a thimble. The thimble has a tubular structure like a large test tube (having diameter and length suitable to occupy

the Soxhlet extraction tube) made of cellulose filter pad. The top of the thimble is plugged with a wad of pure cotton and placed inside the Soxhlet extraction tube, which is then attached to the Soxhlet flask. About 75 ml of anhydrous ether is poured through the sample in the thimble, which percolates through the sample into the flask. The condenser is then attached and the whole assembly is placed in a heating mantle or water bath. Heat is applied just enough to boil the ether. The evaporated ether condenses and falls into the thimble containing the sample and extracts the ether soluble components. When the ether level reaches the siphon outlet level, it is siphoned off into the flask. The distillation process is allowed to continue for about 16 hours. At the end of the extraction period heating is discontinued and the thimble is removed. The ether-extracted sample is used for crude fibre estimation (please refer Practical exercise under Course III). After removing the thimble, ether is distilled off into the Soxhlet tube and poured out before the level reaches the siphoning level. Ether may be distilled off using the assembly B shown in the Fig. When the ether in the flask has reached a small volume, it is transferred into a weighed beaker with repeated rinsing with small portions of ether. The ether in the beaker is evaporated on a steam bath, dry at 100 ^{o}C for 1 hr, cooled and weighed. Crude fat is calculated by:

$$\% \text{ Crude fat} = \frac{\text{Weight of ether-soluble material}}{\text{Weight of sample}} \times 100$$

5.2.2 Protein Estimation

Protein content of food samples is usually determined by the Kjeldahl method, which is based on the determination of the amount of reduced nitrogen present in the sample. The nitrogen compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with sodium hydroxide and the liberated ammonia is absorbed in excess of neutral boric acid solution and titrated with standard acid.

The food sample is digested (destruction of organic matter) with conc. H_2SO_4 in a Kjeldahl flask that is a long neck round bottomed flask. To facilitate digestion and efficient conversion of all the reduced nitrogen into ammonium sulphate, small quantity of a catalyst is added. The catalyst consists of a mixture of selenium dioxide, potassium sulphate and copper sulphate in the ratio of 1:40:8. The digested and cooled sample is made up to a known volume with distilled water and an aliquot of it is distilled in a micro-Kjeldahl distillation apparatus (Figure 5.2).

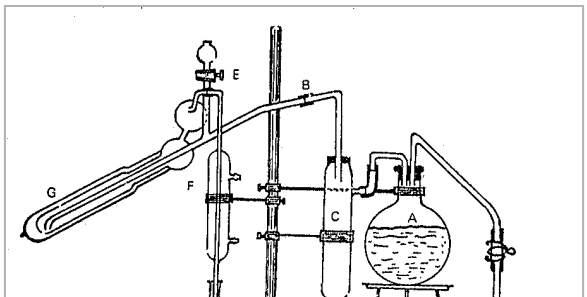


Figure 5.2: Distillation apparatus for micro-Kjeldahl determination of nitrogen *A* steam generator, *B* rubber tubing, *C* steam trap, *D* pinch clamp, *E* funnel, *F* condenser, *G* distillation flask

This is a glass unit with only one short rubber tube joint (B). This rubber joint makes the unit less rigid and reduce the danger of breakage. The steam generator A is a 1-litre round-bottomed flask with a side arm for refilling. The other components of the assembly are the steam trap C, sample delivery funnel E, condenser F and sample holder for steam distillation G.

After initial cleaning of the system with steam, an aliquot of the acid digested sample and strong sodium hydroxide solution are let into the distillation flask and steam distilled. The liberated ammonia is absorbed in a known volume of dilute neutral boric acid solution containing an indicator (mixed indicator) placed below the condenser. On completion of distillation, which takes about 5 min the ammonia absorbed boric acid solution is titrated with 0.01 N HCl. From the titre value, the nitrogen content in the sample is calculated. The function of boric acid in the estimation is only to absorb the liberated ammonia and does not react chemically with it. The titration reaction is:

$$NH_4OH + HCl \rightarrow NH_4Cl + H_2O$$

Calculations:

1000 ml 1 N HCl = 1 g mole of nitrogen = 14 g nitrogen

or 1 ml 1 N HCl = 14 mg nitrogen

Therefore

$$\% \text{ N in the sample} = \frac{\text{Titre x normality of HCl} \times 14 \times 100}{\text{Aliquot of digest taken} \times \text{weight of sample} \times 1000}$$

For calculation of protein content from the nitrogen value, a multiplication factor of 6.25 is usually used. This factor is based on the assumption that plant proteins contain 16 % nitrogen. Therefore, protein content (%) = % N × 6.25.

5.2.3 Pectin Estimation – Calcium Pectate Method

Pectin is an important constituent of fruits. Processing wastes of fruits like citrus peel and pomace, apple pomace etc. are used for commercial, production of pectin. Knowledge of the pectin content of fruits used in jam and jelly manufacture is necessary to calculate the quantity of pectin to be added. Therefore, estimation of pectin content of the raw materials is important.

Pectin extracted from plant materials is usually estimated as calcium pectate. For the estimation, the pectin sample is saponified with alkali and precipitated as calcium pectate from an acid solution by the addition of calcium chloride. The calcium pectate is washed free of chloride, dries and gravimetrically estimated.

Pectin extraction from plant materials: 50 g sample is weighed into a 1000 ml beaker and extracted with 400 ml 0.05 N HCl for 2 hr at 80 – 90^{o}C. Water lost by evaporation is frequently replaced. After cooling the suspension, it is transferred to a 500 ml volumetric flask and made up to volume with distilled water. Subsequently, it is filtered through Whatman No. 4 filter paper.

100-200 ml of the filtrate is taken in a 1 lit beaker and 250 ml distilled water is added to it. The solution is neutralized with 1 N NaOH using phenolphthalein as the indicator and allowed to stand overnight. The next day, 50 ml 1 N acetic acid is added to the solution, stirred and after 5 min, 25 ml 1 N calcium chloride solution is added with stirring. After 1 hr, the solution is boiled for 1-2 min and filtered through a previously dried and weighed (in a covered dish) filter paper circle. The precipitate in the filter paper is washed with almost boiling water till free of chloride. Presence of chloride in the filtrate is tested with silver nitrate solution. The filter paper containing calcium pectate is transferred to the covered weighing dish initially used, dried overnight at 100^{o}C, cooled in a desiccator and weighed. Calcium pectate is calculated as follows.

$$\% \text{ Calcium pectate} = \frac{\text{Weight of calcium pectate} \times 500 \times 100}{\text{ml of pectin extract taken} \times \text{Wt. Of the sample}}$$

The theoretical yield of calcium pectate from pure pectinic acid is about 110%.

5.2.4 Estimation of Tannins

Tannins are widely distributed in fruits and vegetables. They belong to the general group of polyphenols called flavonoids and cinnamic acid derivatives. Tannins are responsible for the undesirable astringency of cashew apple or desirable astringency of apple and apple juice, amla etc. They are responsible for the typical taste and aroma of tea and cocoa. Polyphenols play important role in protecting ascorbic acid in some fruits and can act as a anti-oxidants. Of late, polyphenols have been shown to have several health benefits including prevention of diseases like heart attack, cancer etc.

Tannins can be estimated either by volumetric or by colorimetric method.

Volumetric method

The volumetric method of estimation of tannins is based on the oxidation of tannins by potassium permanganate.

Reagents

1. 0.04 N Potassium permanganate solution (1.40424 g $KMnO_4$ per litre).
2. Indigo carmine solution: 1.5 g indigo carmine in 1 litre water containing 50 ml conc. H_2SO_4.
3. Gelatin solution: Soak 25 g gelatin in saturated sodium chloride solution for 1 hr, heat to dissolve, cool and make up to 1 litre with saturated sodium chloride solution.
4. Acid sodium chloride solution: To 975 ml saturated sodium chloride solution, add 25 ml conc. H_2SO_4.

Procedure

An aliquot of the filtered fruit juice or extract (10 to 20 ml containing about 0.01 g of tannin) is taken in a porcelain dish and 20 ml of the indigo carmine solution is added followed by 500 ml water. From a burette the potassium permanganate solution is added to the dish with vigorous stirring until the colour becomes light green. Then the permanganate solution is added drop wise till the colour changes to bright yellow. The titre value is noted as *A*. Now, another aliquot of the juice or fruit extract (50 ml equal to 10 ml of original juice or extract) is taken in a 250 ml volumetric flask and 25 ml of the gelatin solution is added and made up to volume with the acid sodium chloride solution. It is transferred to a conical flask, a little of filter aid is added, shaken well and filtered. To 50 ml of this filtrate, 20 ml indigo carmine solution is added and titrated with the permanganate solution as done previously. The titre value is noted as *B*.

Calculations

A = Total tannin like materials

B = Non tannin materials

A – *B* = True tannins

One ml of 0.04 N $KMnO_4$ = 0.00168 g tannin (Gallo tannic acid)

$$\% \text{ Tannin} = \frac{(A - B) \times 0.00168 \times 100}{\text{Volume of sample}}$$

Colorimetric method

The colorimetric method for estimation is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline medium. The following reagents are required for the estimation.

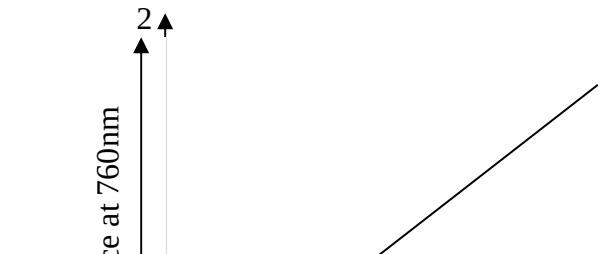
i) Folin – Dennis reagent: A mixture of 100 g of sodium tungstate ($Na_2WO_4.2H_2O$), 750 ml water and50 ml 85% phosphoric acid (H_3PO_4) is refluxed for 2 hr, cooled and diluted to 1000 ml with water.

ii) Saturated sodium carbonate solution: 35 g anhydrous sodium carbonate is dissolved in 100 ml warm (70-80^oC), and cooled. The clear supernatant solution is used for the estimation.

iii) Tannic acid standard solution: Dissolve 100 mg tannic acid in 1 litre water (1 ml = 0.1 mg tannic acid)

Standard curve

To a series of 100 ml volumetric flasks containing 75 ml water, 0 to 10 ml aliquots of the standard tannic acid solution is pipetted followed by 5 ml Folin-Dennis reagent and 10 ml saturated sodium carbonate solution. Make up to volume with water, mix and measure the absorbance (colour) of the solutions after 30 min in a colorimeter or spectrophotometer at 760 nm. A standard curve is prepared by plotting mg tannin in the x axis and the corresponding absorbance on the y axis.



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Tannin estimation in sample

Liquid samples can be used as such. Solid samples (about 5 g) are boiled with water for 30 min, cooled and made up to a known volume.

An aliquot of the prepared sample (containing about 0.1 mg tannin) is used for colour development as in the case of standard. Note the tannin content from the standard curve and the tannin content in the sample is calculated.

Calculations

Weight of sample taken = W ------- g

Sample extract made up to = V ------- ml

Volume taken for colour development = V_1 ------- ml

Tannin content in V_1 ml extract (from standard curve) = a ------- mg

$$\text{Tannin as \% tannic acid} = \frac{a \times V \times 100}{V_1 \times W \times 1000}$$

If liquid sample is taken for estimation as such:

$$\text{Tannin as \% tannic acid} = \frac{\text{mg tannic acid in sample aliquot} \times 100}{\text{ml sample aliquot take} \times 1000}$$

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Differentiate between Proximate analysis and ultimate analysis.

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2. Describe the method for estimating the crude fat content of a food product.

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3. Describe the micro-Kjeldahl method for determination of nitrogen. How the nitrogen value is converted to protein?

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4. Describe the calcium pectate method for pectin estimation

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5. What is the importance of poly phenolic compounds in foods? Explain the volumetric method for tannin estimation.

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5.3 BACTERIOLOGICAL EXAMINATION OF WATER

Large quantities of water are used in the food processing industries for various purposes. Some of the chemical qualities of the water are discussed in another course. Bacteriological quality of water is equally or more important from the public health point of view. The main objectives of bacteriological examination of water required for the food processing industry are to check whether:

i) the water is safe for human consumption,
ii) the water requires treatment,
iii) the treatment followed is adequate.

The following parameters are usually included in the bacteriological examination of water:

i) plate count,
ii) coliform count,
iii) faecal Streptococci test.

Sampling of water: Care should be taken to collect samples of water for bacteriological examination of water. Water should be collected fresh in sterilizes glass bottles and closed properly.

5.3.1 Plate Count

Plate count or total plate count is determined by mixing a known volume of the water sample with a solidifiable culture medium and incubating it for a specified time to allow formation of visible colonies.

Requirements

i) Nutrient agar sterilized in 15 ml quantities in plugged culture tubes.

ii) Sterilized Petri dishes of 10 cm diameter.

iii) Ringer's solution (full strength): Dissolve 9 g sodium chloride, 0.42 g potassium chloride, 0.48 g calcium chloride and 0.2 g sodium bicarbonate in 1000 ml distilled water. Suitably diluted solution is sterilized at 15 psig for 20 min before use.

iv) Dilution bottle each containing 90 ml of quarter-strength Ringer's solution.

v) Sterilized 1 ml and 10 ml pipettes.

Procedure

You have already learnt sample dilution, plating etc. The agar plates are incubated at 37^{o}C for 24 ± 3 hr. After incubation, the colonies formed are counted using a colony counter. The results are expressed as **"plate count per ml at 37^{o}C in 24 hr."** Properly filtered and chlorinated water will not have total plate count in excess of 10 per ml.

5.3.2 Coliform Count

As you have learnt already, presence of coliforms in water indicates the possibility of faecal contamination and hence is strictly monitored. Coliform counts involve three tests viz. i) presumptive test, ii) confirmed test and iii) completed test.

Presumptive test: E-coli is one of the few bacteria, which is able to ferment lactose (lactose broth) with production of acid gas. Therefore, a positive test indicates 'presumptive' evidence for sewage contamination in the water sample. It is only 'presumptive' because several non-faecal bacteria also answer this test. Hence, this test must be 'confirmed'.

Confirmed test: Positive 'presumptive' test sample is plated on eosin-methylene blue (EMB) agar and incubated at 37^{o}C for 24 hr and examined. Typical E-coli colonies will have dark to black centres, button-like in appearance and will often be surrounded by a greenish metallic shine. If positive 'presumptive' sample inoculated into brilliant green lactose bile broth and incubated at 37^{o}C for 24 hr shows gas formation also, it is confirmatory evidence for the presence of E-coli.

Completed test: Completed test for E-coli is performed by transferring a typical colony from EMB agar plate to a nutrient agar slant and lactose broth tube and incubating for 24hr at 37^{o}C. A stained mount of the agar culture is examined under a microscope. A completed test for E-coli should show a pure culture of Gram-negative short rods, and gas formation in the lactose broth tube.

5.3.3 Faecal Streptococci Test

The genus Streptococcus constitutes a diverse group of cocci widely distributed in nature. Some are dangerous pathogens. Streptococci found in faeces are called faecal Streptococci or Enterococci of which Streptococcus faecalis is typical. Streptococci usually occur in pairs of ovoid cocci or in short chains. Unlike many other Streptococci, these grow well in ordinary laboratory media in the presence of bile salt. They produce deep red pin pointed colonies in MacConkey agar and ferment glucose, lactose and mannitol, producing acid but no gas. While most Streptococci are susceptible to heat (55^{o} C for 15 – 20 min), Streptococcus faecalis offers relatively high resistance destructive agents such as heat and can withstand a temperature of 60^{o} C for 30 min. In examining water for the presence of faecal Streptococci, advantage is taken of its relative heat resistance, its ability to form acid in MacConkey broth, and grow in the presence of a concentration of sodium azide sufficient to prove inhibitory to most coliform bacteria. As some anaerobic spore forming bacilli also produce acidity in sodium azide medium, a confirmatory test is also performed. In the confirmatory test, a heavy inoculum from the positive azide tubes is plated on MacConkey agar and incubated at 37^{o} C for 48 hr. The growth of minute pin pointed red colonies is a strong evidence of the presence of faecal Streptococci. On Gram-staining and observing under the microscope, the Streptococci appear as Gram-positive.

5.4 ASSESSMENT OF SURFACE SANITATION

Bacterial counts on utensils, equipment, working surfaces, walls, floors etc., are useful means of assessing the standard of hygiene and the efficiency of cleaning procedures in food factories. The **'Swab Rinse Method'** is useful for the purpose.

For the swab method, pre-sterilized cotton swabs are used. The sterile swabs are dipped in sterile Ringer solution and rubbed over the required surfaces. The rubbed swabs are allowed to stand in sterile Ringer solution for 20-30 min and shaken. This solution is used to make plate counts with yeast extract agar. The Ringer solution inoculated into MacConkey broth should not show

positive test for coliform organisms for well-sanitised surfaces. For bins and large utensils, sterile Ringer solution is used to rinse them. The rinsed solution is plated and inoculated in MacConkey broth as above.

5.5 MICROBIOLOGICAL EXAMINATION OF FOOD SPOILAGE

Microbiological spoilage of foods and its examination is an extremely wide area. Therefore, in this section, microbiological examination of spoilage of canned vegetable products, which is the most important, is discussed.

Canned Products

Canned food spoilage has both monitory loss and public health implications. Microbiological spoilage of canned fruits and vegetables is due to either under processing or post processing contamination (leakage). Under processing is due to insufficient heat treatment resulting in failure to destroy all microorganisms capable of subsequent growth in the product. Leakage is due to contamination of the product, mostly during the cooling process due to faulty seam or damage to the can. Microbiological examination of the spoiled cans help in identifying the cause of spoilage and take remedial measures.

The nature microorganisms associated with spoilage of canned fruits and vegetables is related to the pH of the products. As you have learnt, the spoilage of acidic foods like most fruit products is less critical from safety point of view. The pH of most canned fruits ranges from 3.7 to 4.5. In such products, spoilage is usually caused by aerobic and anaerobic spore formers, though Lactobacilli and Leuconostocs are also encountered occasionally. Tomato is a critical product having pH in the borderline between acidic and low acid foods. Butyric anaerobes and aciduric flat sours are important spoilage organisms of tomato products.

Spoilage in low acid (pH more than 4.5) canned products like vegetables; vegetable soups etc., spoilage due to under processing are caused by thermophiles. Some of these organisms and the type of spoilage are given below.

Bacillus stearothermophilus	*:*	Causes flat sour spoilage
Cl. thermosaccharolyticum	*:*	Causes hard swell
Cl. nigrificans	*:*	Causes sulphur stinkers
Mesophilic spore formers	:	Cause putrefaction

Spoilage due to can leakage is characterised by the presence of a variety of organisms including non-thermophilic organisms like various cocci including Leuconostoc and micrococci. The presence of micrococci and/ or yeasts is almost certain evidence of can leakage.

Examination of can spoilage is an elaborate process. The first step is sampling. If the spoilage is widespread, about 6-12 cans from each batch may be sufficient. Other wise the number of cans required will be quite large. The sampled cans are subjected to the following examinations.

Physical examination: The following parameters are recorded.

i) Name of the product

ii) Code mark

iii) Can size (A1 tall, A2 ½, A10 etc.)

iv) Gross weight

v) Physical condition of the can like mechanical defects, pin holes, swell etc

vi) External seam dimensions

Incubation: The cans are incubated at different temperatures depending on the pH category as below before further examination. Incubation facilitates the multiplication of the surviving organisms, which helps in the subsequent examinations.

Fruit products (pH 4.5 and below): 37^{o} C for 3 days

Vegetable products: Some at 37^{o} C and the remaining at 55^{o} C for 3 days

The cans are examined periodically during incubation and if any cans are swollen, they are removed. After incubation, the cans are cooled before opening.

Opening the Can and Sampling

The cans have to be opened under sterile/ aseptic conditions using sterilizes implements and gas flame. Special type of punches and openers are available for the purpose. Sample is drawn out using sterile glass tubing into a sterile container.

Sample Examination

The sample is used for direct microscopic examination, culturing, pH determination etc. The can under examination is emptied and observed for it's inside appearance and the seam dimensions are examined.

pH determination is important when flat- sour is suspected. As a routine procedure, the net and drained weight of the can is determined and the product quality is visually examined. Test sample should never be tasted. Combustible nature of the gas (hydrogen) in swollen cans can be checked by puncturing the can and directing the gas to a flame. The can interior of hydrogen swelled cans will show heavy corrosion. In the case of sulphur stinker, the can interior will show black or purplish black stain.

Direct Microscopic Examination

The sample taken from the can is smeared on glass slides and stained with methylene blue or carbol fuchsin and examined under the microscope for general morphology of the organisms. Separate slides are also Gram-stained.

The presence of Gram-positive rods suggests under processing while cocci, yeasts etc., suggests leaker spoilage. If spoilage is due to bacteria surviving heat process, not more than one or two types would be present except in gross under processing. In the case of products having pH above 4.0, container leakage would usually show mixed flora. The presence of micrococci and/or yeast is a sure evidence of container leakage. Based on the information, preventive measures such as increasing the process time if under processed or rectification of the can seam if leakage is the cause are undertaken.

Culturing of the can contents is required to identify the microorganisms for further investigations on the spoilage. This is an elaborate process and is not carried out in routine quality control work.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Which are the major parameters included in the microbiological examination of water?

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2. Explain the procedure for determining ‘Plate count’.

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3. Describe the procedure for determining ‘Coliform count’.

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4. Describe the ‘Swab-rinse’ method for assessing surface sanitation.

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5. Describe how a spoiled can is examined for spoilage.

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6. How does microscopic examination of the contents of spoiled can help in preventing subsequent spoilage?

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5.6 LET US SUM UP

As you have learnt, food quality assessment is based on physico-chemical, microbiological and sensory methods. Sensory methods for quality assessment are described under a separate unit.

Analytical food chemistry makes use of physico-chemical methods for determining the composition of foods. In food analysis, proximate analysis is routinely carried out to determine the proximate composition of foods. Proximate composition gives a general idea of the major components of foods. In ultimate analysis, individual compounds of the food are determined.

Many of the analytical methods commonly followed in food analysis are described in the Practical Manuals, which you will be performing. A few of those physico-chemical methods, which could not be covered in the practical Manuals, like determination of 'Crude fat', 'Protein', Pectin' and 'Tannins' are described in this unit.

Crude fat and protein are important parts of the proximate composition of foods. You have already learnt the nutritional importance of fats and proteins. Crude fat includes all fat-soluble constituents like tri-glycerides, phospholipids, sterols, essential oils, fat-soluble vitamins and pigments. Therefore, crude fat extracted from foods is used for determining those constituents also.

Microbiological quality of water used in food processing is very important from the food safety point of view. Potable water should conform to certain microbiological quality standards. The major parameters are plate count, coliform count and faecal Streptococci count. If water from a particular source is found unsafe, it should be treated suitably.

In the food processing industry, surface sanitation of the machinery, utensils etc. used should be thoroughly sanitized before use. The method followed to assess the efficiency of sanitation viz. Swab Rinse method is described in this unit.

Spoilage of foods cause monetary loss and may result in public health hazards. Canned low acid foods are of particular concern. Canned foods are generally spoiled either due to under processing or can leakage. The general procedure

for examining microbiological spoilage in canned foods include, proper sampling of cans, physical observation of the cans, incubation at specified temperatures, opening the cans under sterile/ aseptic conditions, sampling the contents of the can (spoiled food material), microscopic examination and some times culturing the sample. Microbiological examination of spoiled cans helps in preventing their recurrence.

5.7 KEY WORDS

Proximate analysis : Nutritional and biochemical composition.

Ultimate analysis : Analysis of specific compounds and elements.

Crude fat : Lipids and fat-soluble components.

Tannins : Flavonoids and cinnamic acid derivatives.

Presumptive test : Tentative or unconfirmed test.

Flat sour spoilage : Spoilage in cans with acid formation without Bulging.

Hard swell : Can spoilage with hydrogen swell and heavy corrosion.

Under processing : Insufficient heat processing of cans.

5.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Nutritional and biochemical composition
   - Detailed analysis, analysis of individual compounds

2. Your answer should include the following points:

   - Tri-glycerides, phospholipids, essential oils, sterols, fat-soluble vitamins
   - Soxhlet apparatus
   - Ether extraction

3. Your answer should include the following points:

   - Acid digestion
   - Micro-Kjeldahl distillation method
   - % Protein = % Nitrogen × 6.25

4. Your answer should include the following points:

   - Acid extraction of pectin
   - Precipitation as calcium pectate
   - Gravimetric determination

5. Your answer should include the following points:

   - Antioxidant
   - Health benefits

- Oxidation of tannins by potassium permanganate

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Total count
   - Coliform count
   - Faecal Enterococci count.

2. Your answer should include the following points:

   - Nutrient agar
   - Ringers solution
   - Plating
   - Incubation
   - Counting

3. Your answer should include the following points:

   - Presumptive test
   - Confirmed test
   - Completed test

4. Your answer should include the following points:

   - Cotton swab
   - Ringers solution for rinsing
   - Plating
   - Incubation
   - Counting

5. Your answer should include the following points:

   - Can sampling
   - Incubation
   - Can opening
   - Sterile conditions
   - Product sampling
   - Microscopic examination

6. Your answer should include the following points:

   - Spore formers
   - Gram-positive
   - Micrococci
   - Yeasts
   - Under processing
   - Leakage

5.9 SOME USEFUL BOOKS

1. Manual of Methods for the Examination of Water, Sewage and Industrial Wastes (1963) Special Report No. 47, Indian Council of medical Research, New Delhi.

2. National Cannerd Association (1968) Laboratory Manual for Food Canners and Processors, Vol. 1, The AVI Publishing Co., Conneticut, USA.

3. Official Methods of Analysis (1980) Association of Official Analytical Chemists, Washington, DC 20044.

4. Ranganna, S. (2000) Handbook of Analysis and Quality Control for Fruit and Vegetable Products, Tata McGraw-Hill Publishing Co., Ltd., New Delhi.

5. Recommended Methods for the Microbiological Examination of Foods (1958) American Public Health Association Inc., New York.