UNIT 11 SAMPLING TECHNIQUES OF FOOD PRODUCTS

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11.0 OBJECTIVES

After reading this unit, we shall be able to:

- state the importance of sampling in analysis of food products;
- enlist standards and guides on sampling;
- prepare sampling plans;
- describe different sampling techniques; and
- devise ways to draw a representative sample from lots.

11.1 INTRODUCTION

To control food quality and acceptance within satisfactory limits, it is important to monitor the vital characteristics of raw materials, ingredients, and processed foods. This could be done by evaluating all foods or ingredients from a particular lot, which is feasible if the analytical technique is rapid and non-destructive. However, it is usually more practical to select a portion of the total product volume and assume the quality of the selected portion is typical of the whole lot.

Obtaining a portion, or sample, that is representative of the whole is referred to as sampling, and the total quantity from which a sample is obtained is called the population. Adequate sampling technique helps to ensure that sample quality measurements are an accurate and precise estimate of the quantity of the population. By sampling only a fraction of the population, a quality estimate can be obtained more quickly and with less expense and personnel time than if the total population were measured. The sample is only an estimate of the value of the population, but with proper sampling technique, it can be a very accurate estimate.

11.2 SAMPLE COLLECTION

It is important to clearly define the population that is to be sampled. The population may vary in size from a production lot, a day's production, to the contents of a warehouse. Extrapolating information obtained from a sample of a production lot to the population of the lot can be done accurately, but conclusions cannot be drawn from data describing larger populations, such as the whole warehouse.

Populations may be finite, such as the size of a lot, or infinite, such as in the number of temperature observations made of a lot over time. For finite populations, sampling provides an estimate of lot quality. In contrast, sampling from infinite populations provides information about a process. Fig.11.1 represents the sampling for physical and chemical characteristics of food. Regardless of the population type, that is, finite or infinite, the data obtained from sampling are compared to a range of acceptable values to ensure the population sampled is within specifications.

11.2.1 Homogeneous Versus Heterogeneous Populations

The ideal population would be uniform throughout and identical at all locations. Such a population would be homogeneous. Sampling from such a population is simple, as a sample can be taken from any location and the analytical data obtained will be representative of the whole. However, this occurs rarely, as even in an apparently uniform product, such as sugar syrup, suspended particles and sediments in a few places may render the population heterogeneous. In fact, most populations that are sampled are heterogeneous. Therefore, the location within a population where a sample is taken will affect the subsequent data obtained. However, sampling plans and sample preparation can make the sample representative of the population or take heterogeneity into account in some other way.

11.2.2 Manual Versus Continuous Sampling

To obtain a manual sample the person taking the sample must attempt to take a "random sample" to avoid human bias in the sampling method. Thus, the

sample must be taken from a number of locations within the population to ensure it is representative of the whole population. For liquids in small containers, this can be done by shaking prior to sampling. When sampling from a large volume of liquid, such as that stored in silos, aeration ensures a homogeneous unit. Liquids may be sampled by pipetting, pumping, or dipping. However, when sampling grain from a rail car, mixing is impossible and samples are obtained by probing from several points at random within the rail car. Such manual sampling of granular or powdered material is usually achieved with triers or probes that are inserted into the population at several locations. Errors may occur in sampling, as rounded particles may flow into the sampling compartments more easily than angular ones. Similarly, hygroscopic materials flow more readily into the sampling devices than does non hygroscopic material. Horizontal core samples have been found to contain a larger proportion of smaller-sized particles than vertical ones. Continuous sampling is performed mechanically.

Qualitative Characteristics
(e.g. commodity defects)

Inspection of **isolated** lots.	Inspection of a **continuous** series of lots.
E.g., inspection of the aspects of a piece of fruit, or of a can in isolated lots.	E.g., inspection of the aspects of a piece of fruit, or of a can in continuous lots.
To be sampled by **attribute sampling plan for isolated lots.**	To be sampled by **attribute sampling plans for continuous lots.**

Quantitative characteristics
(e.g., compositional characteristics)

Inspection of **isolated** lots

Bulk	**Item**
e.g. fat content of milk in a tank	e.g. sodium content of a dietary cheese.
To be sample by variable sampling plans for isolated lots	Sampling by attributes.

Inspection of **continuous** series of lots

Bulk	**Item**
e.g.: fat content of milk in a tank.	e.g. sodium content of a dietary cheese.
To be sampled by variable sampling plans for continuous series of lots.	To be sampled by attribute sampling plans for continuous series of lots.

Fig. 11.1: Sampling Flowchart for Chemical and Physical Characteristics

11.2.3 Importance of Sample Collection

The reliability of analytical data thus obtained depends on several factors, sampling being the major factor. Current analytical methods require only few grams of food sample to analyze. Thus, it is necessary that a sample be as representative of the population as possible.

There are three basic activities involved in analysis of food products:

- Collection of representative sample.
- Sample preparation.
- Analysis using appropriate methods and instruments.

These activities, although independent in nature, yet can have decisive influence on each other. Furthermore, each of these activities have their own potential sources of variations that contribute to the uncertainty level associated with any analytical result. Thus, care must be taken to identify the sources of variation and minimize or avoid them while accomplishing any activity. On the part of the laboratories, it is therefore necessary to develop a plan for the proper performance of each activity, and then establish quality standards and written procedures in compliance with the standards. Many times, the activity of sampling falls outside the purview of a laboratory's mandate or control. This is especially true in commercial testing laboratories where the "first contact" is the arrival of samples. To improve the overall quality of the analytical process, a laboratory must do all it can to receive appropriate, applicable, defensible samples. The development of appropriate plans will depend upon an understanding of the problems involved in each activity, and then the application of reasonable judgements in seeking solutions.

It should be noted that sampling terminology and procedures used may vary between companies and between specific applications. However, the principles described in this Unit are intended to provide a basis for understanding, developing, and evaluating sampling plans and sample handling procedures for specific applications encountered.

A sample should represent a population as adequately as possible. To ensure proper sampling, the analysts need to be consulted time to time concerning proper sample size, suitable containers for sampling or the use of appropriate preservatives to prevent any spoilage or transformation in a sample before analysis. One common cause of lack of precision or lab-to-lab variation in analytical results for a particular population can be traced back to erroneous sampling. For example, in case of grapes, a laboratory sample size of meager 3 kg berries represents the whole population of > 10000 kg in 1 hectare vineyard area. Thus, if the sample collected is not representative, then there will be sample-to-sample variation in results. When significant difference in results occurs among laboratories which have supposedly analyzed the same sample, a serious conflict may arise questioning the competence and credibility of the laboratories. Many of these situations can be avoided if samples are collected according to a rational plan that gives some assurance that the sample delivered to the laboratory represents the composition of the parent lot.

There are at least two ways to measure a given lot of goods: one, that we often assume to be the "proper" way, is to find its "true value", by which we mean its average value. The other way, often discovered accidentally as a result of

"poor" sampling, is to measure its variability. So called proper sampling of drug dosage forms, for example, may involve compositing 20 tablets, by which the majority of the tablets could be used to dilute and conceal the fact that several of them are severely sub- or super- potent. Similarly, two lots of grain may have been purposely, but ineffectively, mixed in an attempt to reduce the average level of a contaminant. Sampling that led to the laboratory finding inconsistent results would reveal the attempt to dilute an illegal product.

11.2.4 Errors in Analytical Results due to Improper Sampling

Few studies have been conducted on the distribution of error among the three activities: sampling, sample preparation, and analysis. In one such study, which involved analysis of 20 nanogram/gram concentration of aflatoxin in a lot of peanuts, the error contributed by the sampling step was as high as 67% of the total variance, in comparison to 20% and 13% errors contributed by the analyst and the analytical procedure, respectively. In a field study conducted at the National Research Centre for Grapes, Pune, the pesticide residues in grape samples analyzed in 15 individual grape bunches collected out of 1 acre area showed above 50% sampling-induced variations. The results of such experimentations are not unusual and it illustrates the proportion of error that can be attributed to sampling. For peanuts, the distribution of aflatoxin can vary widely, with a few peanuts accounting for most of the contamination. Similarly, in case of the field sampling of grapes, the pesticide residues might have deposited in variable concentrations in different grape bunches and thus when they were analyzed separately, showed variable results. The important point in these examples is to show that sampling error can play a very significant part in the overall error in the analytical system.

11.2.5 Risks Associated with Sampling

There are two types of risks associated with sampling. Both should be considered when developing a sampling plan. The consumer risk describes the probability of accepting a poor quality population. This should happen rarely (<5% of the lots) but the actual acceptable probability of a consumer risk depends on the consequences associated with accepting an unacceptable lot. These may vary from major health hazards and subsequent fatalities to a lot being of slightly lower quality than standard lots. Obviously, the former demands a low or no probability of occurring whereas the latter would be allowed to occur more frequently. The second risk i.e., vendor risk is the probability of rejecting an acceptable product. As with consumer risk, the consequences of an error determine the acceptable probability of the risk. An acceptable probability of vendor risk is usually 5-10%.

11.3 SAMPLING STANDARDS

Data obtained from an analytical technique are the result of a stepwise procedure from sampling, to sample preparation, laboratory analysis, data processing, and data interpretation. There is a potential for error at each step and the uncertainty, or reliability, of the final result depends on the cumulative errors at each stage. Variance is an estimate of the uncertainty. The total variance of the whole testing procedure is equal to the sum of the variances associated with each step of the sampling procedure and represents the precision of the process. Precision is a measure of the reproducibility of the data. In contrast, accuracy is a measure of how close the data are to the true value. The most efficient way to improve accuracy is to improve the reliability

of the step with the greatest variance. Frequently, this is the initial sampling step. The reliability of sampling is dependent more on the sample size than on the population size. The larger the sample size, the more reliable the sampling. However, sample size is limited by time, cost, sampling methods, and the logistics of sample handling, analysis, and data processing.

It should be noted that sampling terminology and procedures used may vary between companies and between specific applications. Several standards and recommendations provide the ways and means to sample a particular lot.

ISO specifies various standards and guidelines for drawing samples and data interpretation for samples. Few such standards related to food are mentioned in Table 11.1.

Table 11.1: Standards and Guides of Sampling

ID of Standard/Guide	Title of Standard/Guide
ISO 2854 : 1976	Statistical Interpretation of data- techniques of estimation and tests relating to means and variances.
ISO 2859-0 : 1995	Sampling procedures for inspection by attributes-Part 0 ; Introduction to the ISO 2859 Attribute sampling system
ISO 2859-1 : 1999 / IS 2500 (Part I) : 1989	Sampling procedures for inspection by attributes-Part 1; Sampling plans indexed by Acceptable Quality Level (AQL) for lot-by-lot inspection.
IS 2500 (Part II) : 1965	Sampling inspection procedures.
ISO 2859-2 : 1985	Sampling procedures for inspection by attributes- Part 2; Sampling plans indexed by Limiting Quality (LQ) for isolated lot inspection.
ISO 3494 : 1976	Statistical interpretation of data – Power of tests relating to mans and variances.
ISO 3951 : 1989	Sampling procedures and charts for inspection by variables for per cent non-conforming.
ISO 5725-1 : 1994	Application of statistics- Accuracy (trueness and precision) of measurement methods and results- Part 1; General principles and definitions.
ISO 7002 : 1986	Agricultural food products-Layout for a standard method of sampling a lot.
ISO 8423 : 1991	Sequential sampling plans for inspection by variables for per cent non-conforming (known standard deviation).
ISO 8422 : 1991	Sequential sampling plans for inspection by attributes.
ISO/ TR 8550 : 1994	Guide for the selection of an acceptance sampling system, scheme or plan for inspection of discrete items in lots.
ISO 10725 : 2000	Acceptance sampling plans and procedures for the inspection of bulk material.

ISO/ FDIS 11648/1	Statistical aspects of sampling from bulk materials – Part 1; General principles.
ISO/ DIS 14560	Acceptance sampling procedures by attributes – specified quality levels in non-conforming items per million.
Other IS specifications	Specifications published by BIS pertaining to individual products contain the details of lot size and corresponding number and quantity of samples to be drawn and tested for conformance.

Since all standards are subject to revision, parties to agreements based up on these guidelines should ensure that the most recent editions of the standards are always applied.

An example for flour sampling as per AOAC method is presented here. The AOAC Method 925.08 (6) describes the method for sampling flour from sacks. The number of sacks to be sampled is determined by the square root of the number of sacks in the lot. The sacks to be sampled are chosen according to their exposure. The samples that are more frequently exposed are sampled more often than samples that are exposed less. Sampling is done by drawing a core from a corner at the top of the sack diagonally to the center. The sampling instrument is a cylindrical, polished trier with a pointed end. It is 13 mm in diameter with a slit at least one third of the circumference of the trier. A second sample is taken from the opposite corner in a similar manner. The cores are stored for analysis in a clean, dry, airtight container that has been opened near the lot to be sampled. The container should be sealed immediately after the sample is added. A separate container is used for each sack. Additional details regarding the container and the procedure also are described below.

Title 21 CFR specifies the sampling procedures required to ensure that specific foods conform to the standard of identity. In the case of canned fruits, 21 CFR 145.3 defines a sample unit as "container, a portion of the contents of the container, or a composite mixture of product from small containers that is sufficient for the testing of a single unit". Furthermore, a sampling plan is specified for containers of specific net weights. The container size is determined by the size of the lot. A specific number of containers must be filled for sampling of each lot size. The lot is rejected if the number of defective units exceeds the acceptable limit. For example, out of a lot containing 48,001 to 84,000 units, each weighing 1 kg or less, 48 samples should be selected. If six or more of these units fail to conform to the attribute of interest the lot will be rejected. Based on statistical confidence intervals, this sampling plan will reject 95% of the defective lots examined, that is, 5% consumer risk.

Table 11.2: Minimum Number of Primary Samples to be taken from a Lot
(Ref: CODEX doc. CAC/GL 33)

Name of Commodity	**Minimum Number of Primary Samples to be taken from a Lot**
1. Meat and Poultry	
a) Non-suspect lot	1
b) Suspect lot	Determined according to Table 11.3

2. Other products	
a) Products packaged in bulk, which can be assumed to be well mixed or homogeneous.	1 (A lot may be mixed by grading or manufacturing process)
b) Products packaged in bulk, which may not be well mixed or homogeneous.	For products comprised of large units, the minimum number of primary samples should comply with the minimum number of units required for the laboratory sample.
Either Weight of lot, kg	
<50	3
50-500	5
>500	10
or, Number of cans, cartons, containers in the lot	
1-25	1
26-100	5
> 100	10

Table 11.3: Number of randomly selected primary samples required for a given probability of finding at least one non-compliant sample in a lot of meat or poultry for a given incidence of non-compliant residue in the lot

(Ref: CODEX doc. CAC/GL 33)

Incidence of Non-compliant Residues in the Lot	Minimum number of samples (n_o) required to detect a non- compliant residue with a probability of		
%	**90%**	**95%**	**99%**
90	1	-	2
80	-	2	3
70	2	3	4
60	3	4	5
50	4	5	7
40	5	6	9
35	6	7	11
30	7	9	13
25	9	11	17
20	11	14	21
15	15	19	29
10	22	29	44
5	45	59	90
1	231	299	459
0.5	460	598	919
0.1	2302	2995	4603

Notes:

a) The table assumes random sampling.

b) Where the number of primary samples indicated in Table 11.2 is more than about 10% of units in the total lot, the number of primary samples taken may be fewer and should be calculated as follows:

$$n = \frac{n_o}{1 + (n_o - 1) / N}$$

Where, n = minimum number of primary samples to be taken

n_o = number of primary samples given in Table 11.2

N = number of units, capable of yielding a primary sample in the lot.

c) Where a single primary sample is taken, the probability of detecting a non-compliance is similar to the incidence of non-compliant residues.

d) For exact or alternative probabilities, or for a different incidence of non-compliance, the number of samples to be taken may be calculated from:

$$1 - p = (1 - i)^n$$

Where, p is the probability and i is the incidence of non-compliant residue in the lot (both expressed as functions, not percentages) and n is the number of samples.

Table 11.4: Sampling Procedure and Minimum Amount (composite/bulk) to be Sampled from lots

Crop Type	Sampling Procedure	Example	Minimum Quantity
Root, tuber and bulb vegetables	Take samples from all areas of the crop. Remove as much adhering soil as possible from samples but do not wash.	Beet (red, sugar, fodder), onions, parsnips, potatoes, sweet potatoes, turnips	5 kg (and not less than 5 items)
	(**Note:** In some cases, where leaf parts are used as stock feed, they may need to be sampled separately).	Carrots, radish, spring onions.	2 kg
	Take the sample from all areas of the crop. Sample parts of the crop exposed to the spray and also those apparently protected by foliage.	Brassica (cabbage, cauliflower, broccoli, kohlrabi, curly kale).	5 kg (and not less than 5 items)
Leafy, stem, fruiting and legume vegetables	Remove as much soil as possible from crops such as celery, but do not wash.	Asparagus, brussel sprouts, celery, chicory, lettuce, spinach, turnip tops	2 kg
		Cucumber, melon, squashes, eggplant	5 kg (and not less than 5 items)
		Peppers, tomatoes, gherkins	2 kg
		Beans, peas, etc. (with pods)	2 kg
All tree and bush fruit, including vines, small fruits and berries	Select fruit from all parts of the tree/bush, high and low, and from both sides of the row, and select fruits according to abundance whether in each segment or	Apples, citrus, peaches, pears	5 kg

	the whole tree/bush. More fruit should therefore be selected from the more densely laden parts of the crop.		
	Sample parts of the crop exposed to the spray and also those apparently protected by foliage.	Cherries, nuts, olives, plums.	2 kg
	Take large and small fruits, perfect or slightly blemished, but not so small or blemished that they would not normally be saleable.	Bush fruit (all types), grapes, strawberries	2 kg
Cereal Grains	Cut not less than ten small areas (approximately 0.1 m^2) chosen randomly from all areas of the crop. Cut stalks about 10 cm above the ground. Remove grain from the straw.	Maize (grain and cobs)	2 kg
Oil Seeds	Collect the heads when they have reached the stage of maturity at which they are normally harvested and if convenient thresh to remove the seeds.	Sesame, canola, soybeans, sunflower	1 kg of seeds

Table 11.5: Description of Primary samples and min. size of laboratory sample

(Ref: CODEX doc. CAC/GL 33)

S. No	Commodity Classification	Examples	Nature of Primary Samples to be taken	Minimum Size of Lab Sample
1. Primary food commodities of animal origin				
1.1	Large mammals *Whole or half carcass* *Usually 10 kg or more*	Cattle Sheep Pigs	Whole or part of diaphragm, supplemented by cervical muscles, if Necessary	0.5 kg
1.2	Small mammals *Whole carcass*	Rabbits	Whole carcass or hind quarters	0.5 kg, after removal of skin and bone
1.3	Mammal meat parts, loose fresh/chilled/frozen Packaged or other wise	Quarters Chops Steaks Shoulders	Whole unit(s),or a portion of a large unit	0.5 kg, after removal of bone
1.4	Mammal meat parts, bulk frozen	Quarters Chops	Either a frozen cross-section of a container or the whole (or portions of individual) meat parts	0.5 kg, after removal of bone
2. Poultry fats				
2.1	Birds, at slaughter whole or part-carcass	Chickens Turkeys	Units abdominal fat from at least three birds	0.5 kg

2.2	Bird meat parts	Legs Breast – muscle	Either visible fat, trimmed from units	0.5 kg
			Whole units or portions where fat is not trimmable	2 kg
2.3	Bird fat tissue in bulk		Units taken with a sampling device from at least three portions	0.5 kg
2.4	Poultry eggs		Whole eggs	12 whole eggs
2.5	Liquid, frozen or dried egg products		Whole units	0.5 kg
3. Processed foods of animal origin				
3.1	Mammal or bird comminuted, cooked, canned, dried, rendered or otherwise processed products including multi ingredient products	Ham sausage / Minced beef / Chicken paste	Packaged units or a representative cross section from a container or units (including juices, if any) taken with a sampling device	0.5 kg or 2 kg if fat content < 5%
3.2	Liquid milks, milk powders, ice creams etc.		Packaged units	0.5 L or 0.5 kg
3.3	Cheese	Units > 0.3 kg	Whole unit	0.5 kg
		Units < 0.3 kg	Whole unit	0.3 kg
4. Herbs		Fresh	Whole units	0.5 kg
		Dried	Whole units	0.1 kg
5. Feed commodities of plant origin				
5.1	Legume animal feeds and other forages and fodders		Whole units	1 kg (at least 10 units)
5.2	Straw, hay and other dry products		Units taken with a sampling device	0.5 kg (at least 10 units)
6. Processed foods of plant origin				
6.1	Products of high unit value		Packages or units taken with a sampling device	0.1 kg
6.2	Solid products of low bulk density	Hops Tea	Packages or units taken with a sampling device	0.2 kg
6.3	Other solid products	Bread Flour Dried fruits Vegetable	Packages or units taken with a sampling device	0.5 kg
6.4	Liquid products	Oils Juices	Packages or units taken with a sampling device	0.5 L or 0.5 kg

Check Your Progress Exercise 1

Note: a) Use the space below for your answers.

b) Check your answers with those given at the end of the unit.

1) What is the importance of sample collection in analysis of food products?

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2) What are homogenous and heterogeneous populations?

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11.4 THE SAMPLING PLAN

11.4.1 Understanding a Sample Plan

Sampling is generally done for a specific purpose and the purpose may indeed suggest or dictate the nature of any sampling plan. The International Union of Pure and Applied Chemistry (IUPAC) defines a sampling plan as "a predetermined procedure for the selection, withdrawal, preservation, transportation, and preparation of the portions to be removed from a lot as samples". A sampling plan should be a well-organized document that establishes the required procedures for accomplishing the program's objectives. It should address the issues of who, what, where, why, and how. The primary aim of sampling is to obtain a sample, subject to constraints on size, that will satisfy the sampling plan specifications. A sampling plan should be selected on the basis of the sampling objective, the study population, the statistical unit, the sample selection criteria, and the analysis procedures. Factors determing the choice of a sampling plan are enlisted in Table 11.6. The two primary objectives of sampling are often to estimate the average value of a characteristic and determine if the average value meets the specifications defined in the sampling plan. The presence of a well designed plan is important because it provides a consistent model to guide people performing the sampling activity, and it serves as a reminder of the important elements in this part of the overall sample analysis program.

Table 11.6: Factors Affecting Choice of Sampling Plans

Factors to be considered		Questions
Purpose of inspection	a	Is it to accept or reject the lot?
	b	Is it to measure the average quality of the lot?
	c	Is it to determine the variability of the product?
Nature of Product	a	Is it homogeneous or heterogeneous?
	b	What is the unit size?
	c	How consistently have past populations met specifications?
	d	What is the cost of the material being sampled?
Nature of the test method	a	Is the test critical or minor?
	b	Will someone become sick or die if the population fails to

		pass the test?
	c	Is the test destructive or non-destructive?
	d	How much does the test cost to complete?
Nature of the population being investigated	a	Is the lot large but uniform?
	b	Does the lot consist of smaller, easily identifiable sublots?
	c	What is the distribution of the units within the population?

11.4.2 Statistical Approaches

In many sampling programs, statistical approaches are not given the requisite attention. Percentage sampling systems that specify a fixed percentage of a lot, say 5 or 10%, do not provide the quality protection that is often assumed. Statistical sampling theory furnishes the means to analyze the relationship between a lot of goods and the samples that are drawn from it. It can be used to estimate population measure, or "parameters," such as variance and correlation, from knowledge of corresponding samples quantities. The importance of sampling is recognized in ISO 17025, (this standard will be discussed in detail in Course 5, Block 3) which requires that test reports make reference to the sampling procedure used by the laboratory or the submitting body.

Check Your Progress Exercise 2

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

1) Sampling plan is very important in sampling. Why?

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2) Mention the purposes that are served by a sampling plan.

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11.5 SAMPLING TECHNIQUES/METHODS

There are several sampling methods/techniques in common use. These are probability sampling, non-probability sampling, bulk sampling, and acceptance sampling. These are described in brief below:

11.5.1 Probability Sampling

Probability sampling is used when a representative sample is desired, and uses principles of statistical sampling and probability i.e. elimination of human bias.

It is a random selection approach that tends to give each unit an equal chance of being selected.

Simple random sampling requires that the number of units in the population be known and each unit is assigned a number. A specific quantity of random numbers between one and total number of population units is selected. Sample size is determined by lot size and potential impact of a consumer or vendor error. Units corresponding to the random numbers are then analyzed as an estimate of the population.

Systematic sampling is used when a complete list of sample units is not available, but when samples are distributed evenly over time or space, such as on a production line. The first sample is selected at random and then every *n*th unit after that.

Stratified sampling involves dividing the population into overlapping subgroups so that each subgroup is as homogenous as possible. Group means, therefore, differ from each other as much as possible. Random samples are then taken from each subgroup. The procedure provides a representative sample because no part of the population is excluded and it is less expensive than simple random sampling.

Cluster sampling entails dividing the population into clusters or subgroups so that cluster's characteristics are as identical as possible, that is, the means are very similar to each other. Any heterogeneity occurs within each cluster. Clusters should be small and having a similar number of units in each cluster. The clusters are sampled randomly and may be either totally inspected or sub-sampled for analysis. This sampling method is more efficient and less expensive than simple random sampling, if populations can be divided into homogenous groups.

Composite sampling is used to obtain samples from bagged products such as flour, seeds, and larger items in bulk. Two or more samples are combined to obtain one sample for analysis that reduces differences between samples. For example, FDA composite 12 and at least six subsamples, respectively, for the sample to be analyzed for compliance with nutrition labeling regulations.

11.5.2 Non-probability Sampling

Non-probability sampling is used when it is not possible to collect a representative sample, or a representative sample is not desired. For example, in case of adulteration such as rodent contamination, the objective of the sampling plan may be to highlight the adulteration rather than collect a representative sample of the population. The sample collector uses judgement rather than statistical considerations in the selection of the sample. The unusual or unexpected characteristics in a population could be selected to be identified. This type of sampling is done in many ways, but in each case the probability of including any specific portion of the population is not equal because the investigator selects the samples without estimating sampling error.

Judgement sampling is solely at the discretion of the sampler and therefore is highly dependent on the person taking the sample. This method is used when it is the only practical way of obtaining the sample. This method may present a better estimate of the population than random sampling if sampling is done by an experienced individual and limitations of extrapolations from the results are understood.

Convenience sampling is performed when ease of sampling is the key factor. The first pallet in a lot or the sample that is most accessible is selected. This type of sampling will not be representative of the population, and therefore is not recommended.

Restricted sampling may be unavoidable when the entire population is not accessible. For example, if sample is to be taken from a loaded truck, but the sample is not a representative of the entire population.

Quota sampling is the division of a lot into groups representing various categories, and samples are then taken from each group. This method is less expensive than random sampling but also is less reliable.

11.5.3 Types of Sampling

A) Bulk sampling

Bulk sampling involves the selection of a sample from a lot of material that does not consist of discrete, identifiable or constant units. Sampling may be performed in static or dynamic situations. Bulk sampling poses special problems requiring certain decisions to be made: the number of increments to be taken, the size of the increments, from where in the pile or stream they should be drawn, the sampling device to be used, and how to reduce the increments taken to a reasonable size of sample for delivery to the laboratory.

B) Acceptance sampling

Acceptance sampling differs from the previous types and involves the application of a predetermined plan to decide whether a lot of goods meet defined criteria for acceptance. The risks of accepting "bad" or rejecting "good" lots are stated in conjunction with one or more parameters, for example, quality indices of the plan. Statistical plans can be designed to regulate the probabilities of rejecting good lots or accepting bad lots.

Refer Tables 11.2 and 11.3 in the annexure.

There are two broad categories of acceptance sampling: sampling by attributes and sampling by variables.

Sampling by attributes

In sampling by attributes, the unit of product is classified as defective or non-defective, or the number of defects in a unit of product is counted with respect to a given requirement. Or, the sampling is performed to decide on the acceptability of a population based on whether the sample possesses a certain characteristic, for example, *Clostridium botulinum* contamination in canned goods. An example of net weight determination may serve to explain the differences between the two categories. In attribute sampling, each unit that weighs 1 pound or more is accepted, and each unit that weighs less than 1 pound is rejected. If the number of rejects exceeds a predetermined number, the lot is rejected. If the number of rejects is less than the predetermined number, the lot is accepted.

Sampling by variables

In variable sampling, sampling is performed to estimate quantitatively the amount of a substance (e.g., salt) or a characteristic (e.g., color) on a continuous scale. The estimate obtained from the sample is compared with an

acceptable value (i.e., previously determined) and the deviation measured. This type of sampling usually produces data that have a normal distribution such as in the per cent fill of a container and total solids of a food sample. In general, variable sampling requires smaller sample size than attribute sampling and each characteristic should be sampled for separately when possible.

11.5.4 Operating Characteristic (OC) Curves

Operating Characteristic (OC) curves are used extensively in acceptance sampling. The OC curve shows the relationship between the quality and the per cent of lots expected to be acceptable for the quality characteristic inspected. In other words, the OC curve is a graph of lot defectives against the probability that the sampling plan will accept the lot. Fig. 11.2 depicts OC curves for an ideal sampling plan.

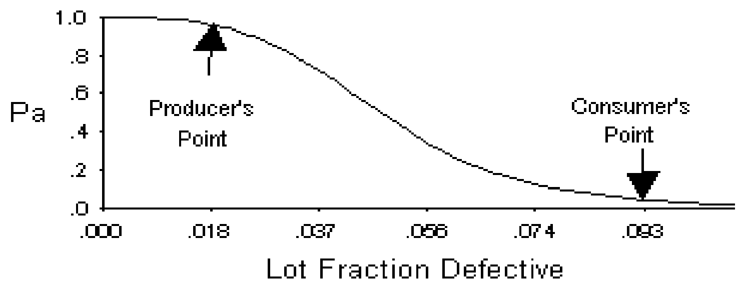

Fig. 11.2: Operating Characteristic Curve

The Operating Characteristic (OC) curve shows the probability of acceptance, Pa, for any level of lot quality. On the horizontal axis is the quality characteristic. This OC curve enables you to evaluate the probability of acceptance for any true lot quality level-on a what-if basis. This way, you can design sampling plans that **perform** the way you want.

We can interpret the curve according to this example:

1) If the lot quality is 0.093 fraction defective, then the probability of acceptance, Pa, is 0.05.

2) If the lot quality is 0.018 fraction defective, then the probability of acceptance, Pa, is 0.95.

11.5.5 Requirements of Good Sampling Methods

Samples are useful for their intended purpose when they are taken in a manner consistent with generally recognized good sampling techniques and good sampling practices. This requires the following:

- Inspection of the lot before sampling.
- Use of suitable sampling devices for the particular commodity and type of sample desired.
- Use of suitable containers to hold the sample.
- Maintenance of the integrity of the sample and associated records.
- Use of adequate precautions in preserving, packing and delivery of the sample to the lab in a timely manner.

- Provision of appropriate storage conditions for the sample both prior to and following analysis.

All of these factors, along with others such as cost versus benefits analysis, and a review of program objectives and regularity requirements, are to be assessed and brought together in a sampling plan that serves as a guide to management, as well as to operating personnel as a firm plan to achieve quality in sampling.

11.5.6 Cost of Sampling

The attention of users is drawn upon relation between the efficiency and size of sample. For a given Acceptable Quality Level (AQL), the smaller the sample size, the smaller the cost of sampling, but the worse the efficiency, that is the risk to wrongly accepting a lot increases and worsens the damage in trade.

11.5.7 Problems in Sampling

Analytical data never are more reliable than the sampling technique. Sampling bias, due to non-statistically viable convenience, may compromise reliability. Errors also may be introduced by not understanding the population distribution and subsequent selection of an inappropriate sampling plan.

Unreliable data also can be obtained by non-statistical factors such as poor sample storage resulting in sample degradation. Samples should be stored in a container that protects the sample from moisture and other environmental factors that may affect the sample (e.g., heat, light, air). To protect against changes in moisture content, samples should be stored in an airtight container. Light sensitive samples should be stored in containers made of opaque glass, or the container wrapped in aluminum foil. Oxygen sensitive samples should be stored under nitrogen or an inert gas. Refrigeration or freezing may be necessary to protect chemically unstable samples. However, freezing should be avoided when storing unstable emulsions. Preservatives (e.g., mercuric chloride, potassium dichromate, and chloroform) can be used to stabilize certain food substances during storage.

Mislabeling of samples causes mistaken sample identification. Samples should be clearly identified by markings on the sample container in a manner such that markings will not be removed or damaged during storage and transport. For example, plastic bags that are to be stored in ice water should be marked with water-insoluble ink.

If the sample is an official or legal sample the container must be sealed to protect against tampering and the seal mark easily identified. Official samples also must include the date of sampling with the name and signature of the sampling agent. The chain of custody of such samples must be identified clearly.

11.6 THREE CLASS SAMPLING PLAN

Another type of sampling used frequently by regulatory agencies to determine acceptance or rejection of a lot (often defined as the quality of product produced under essentially the same conditions but representing no more than one day's production) is the three-class sampling plan. This approach is often used when assessing microbiological contamination of foods. In this case, "n" is the number of samples, usually selected at random from the lot, the

numerical value “m“ represents acceptable concentrations, the numerical value “M” represents un-acceptable concentrations, and “c” is the maximum allowable number of marginally acceptable sample units such that if this number is exceeded, the lot is considered as un-acceptable. While “m” separates sample units of acceptable quality from those of marginally acceptable quality, “M” separates sample units of marginally acceptable quality from those of defective quality.

For enforcement purposes, the sampling technique used should be the same as the sampling technique used to set the standard. For example, minimum reportable limits for particles are based on composite samples and not on individual lots.

11.7 PREPARATION OF SAMPLING PLANS

The development of quality sampling plans is a science in itself and has been given consideration by a number of organizations. One plan format that deserves serious consideration, developed by the International Organization for Standardization, is shown with comments in ISO/TC 34, ISO/DIS 7002.2, “Agricultural food products- Layout for a standard method of sampling from a lot “(1988).

It can serve as a starting point or check list for developing a sampling plan for most commodities. The title and headings from sections in the monograph are as below:

11.7.1 Model Sampling Plan

Agricultural food products- Layout for a standard method of sampling from a lot.

1) Title (short but appropriate for index identification)
2) Introduction (describing the purpose of the plan)
3) Scope (describing the breadth of coverage of the plan)
4) Field of application (products to be covered; where sampling will be done)
5) References (documents, the validity of the plan with reference to other requirements)
6) Definitions (specific terms associate with a particular matrix)
7) Principle (statistical basis of the method of sampling)
8) Administrative arrangements
   a) Sampling personnel
   b) Representation of parties concerned
   c) Health, safety and security precautions
   d) Preparation of the sampling report
9) Identification and inspection of the lot prior to sampling (important in survey sampling for identification, condition of the lot and selection of method of sampling).
10) Sampling equipment and ambient conditions (proper tools such as use of sterile equipment for aseptic sampling).

11) Sample containers and packing (essential to prevent contamination and damage during shipment or storage).

12) Sampling procedures (as dictated by the plan objectives).

   a) Sampling size (adequate for all analytical testing to be done. Refer Tables 11.2 to 11.4 in Annexure)

   b) Taking the sample.

   c) Preparation of bulk samples (Table 11.3) and reduced samples (Table 11.4).

   d) Selection of samples of pre-packaged products.

13) Packing, sealing and marking of samples and sample containers (identification of units and to establish chain-of-custody).

   a) Filling and sealing sample containers.

   b) Labeling or marking (including signature of sampling personnel).

   c) Packing samples for storage or transportation.

14) Precautions during storage and transportation of samples.

15) Sampling report

   a) Administrative details.

   b) Details of unit packs or enclosure containing the lot.

   c) Material samples.

   d) Marking and sealing of samples.

16) Annexure (supplemental information, if necessary).

Check Your Progress Exercise 3

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

1) Mention some sampling methods/techniques related food products?

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2) What is probability sampling?

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3) Describe advantages and disadvantages of the plans- Sampling by attributes and sampling by variables?

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4) Write down the application flow of three- class sampling plan?

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5) Mention some basic requirements of good sampling methods?

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6) How much quantity of sample is required for the following items?

   Root and bulb vegetables, Cereal grains, Poultry eggs, Liquid milk, Liquid products.

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11.8 SUB SAMPLING FOR ANALYSIS AND TAKING THE TEST PORTION

If the test portion analyzed does not represent the sample or the lot from which it was taken, in that case, even the best analysis could give misleading information. Distortions introduced at this point will carry through the path of analysis and adversely affect the final results and the conclusions drawn from them. There are generally two choices in analytical sub sampling:

- Preparation of a composite laboratory sample (if multiple units are submitted for analysis).
- Examination of individual units.

11.8.1 Composite Lab Sample Preparation

A composite lab sample is one in which the individual units or representative portions of units are mixed to form a uniform mixture. Portions are then taken

from the composite for analysis. Compositing saves analytical time and in some types of contract testing it may be the procedure specified. If the results indicate that there may be a problem, it will likely be necessary to go back and analyze individual samples. Compositing is not the procedure of choice when there is a chance that an individual unit that constitutes a public health or safety threats will not be detected (there are some exceptions) or where a unit at or outside of tolerance level will not be detected because of matrix dilutions. Multiple unit lab sampling is indicated when the possible range of values among individual units is considered significant or it is desirable to establish the variability of the lot.

Refer to Tables 11.4 and 11.5 for the details commodities and quantity of sample required.

11.8.2 Opinions of Experts

You den and Steiner (Statistical Manual of the AOAC, AOAC International, Arlington, VA, p 41) observed that, "Many materials are notoriously difficult to sample. Often the variability among samples is the controlling factor in the confidence placed in the analytical result." They note further that

A mistake sometimes made is to composite several samples and then to run repeat determinations on this composite sample. The analyst may be happy with several results that are in close agreement because only the analytical error is involved in the results. And some may put their faith in the result admittedly, if the individual samples were of the same weight and properly mixed, the same average will result whether the samples are analyzed individually or repeats are made on the composition. Using the composite sample effectively conceals the between-sample variation. It should be mandatory to run the samples individually, for only by doing so will anybody be in a position to make any statistical statements about the results, no matter how good the analytical procedure.

Somewhat similar view of sub sampling for analysis is expressed in an article published in Chemical and Engineering news by an ad hoc sub committee of The American Chemical society for "Dealing with the Scientific Aspects of Regularity Measurements."

This report observes that *the number of samples to be analyzed in a given situation usually is limited by the resources available for collection of samples or for their analysis. However, the reliability of the result generally increases with the square root of the number of samples analyzed. For this reason, analysis of multiple samples are preferred over single samples since, single samples give no information on the homogeneity of the lot that was sampled. In addition, for single samples, the sampling error is also confounded with the analytical error. As a result, if the total number of determinations must be fixed, multiple independent single samples are preferred over replicate aliquots per a single random sample. In any case, the sampling decisions should be a priori decision and should be based on the question at issue.*

In addition to the number of sub samples taken for analysis, it is essential that each be prepared in a way that achieves homogeneity and is handled in a manner that prevents alteration from the original composition. Obviously, failure to prepare a homogeneous sub sample at this point will affect the results of the analysis regardless of the method used.

11.9 SAMPLE PREPARATION FOR ANALYSIS

Every type of material that is to be prepared for analysis presents its own practical difficulties. The requirements for suitable sample preparation are dictated by the consistency and the chemical characteristics of the analyte and the matrix, and by the distribution of the analyte in the sample. Even seemingly homogeneous materials such as liquids may be subject to sedimentation or stratification. Thus, vigilance and care are the watch words to ensure homogenity.

11.9.1 Precautions to be followed while Preparing a Sample for Analysis

Mixing: Single phase liquids can generally be mixed, stirred, shaken or blended. Dry particulate materials can be reduced in the volume by coning and quartering, by rolling and quartering, or by the use of a splitter, such as a refill. A variety of implements and machines are available for sample disintegration, such as mills, grinders and cutters. Care in their use is necessary to prevent loss of dust or change in composition through partial separation of components. Screening can be used to improve the efficiency of particle size reduction, followed by mixing to attain homogeneity. Sampling errors can occur even in well mixed particulate mixtures especially in trace analysis if the particles differ appreciably in size or physical properties.

Cleanliness of equipment used in process

Every piece of equipment used in the preparation of a sample must be examined critically to ensure their cleanliness, so that they do not contaminate or decompose or cause any physical loss of the sample while processing. Grinders were mentioned above as contributing to the loss of finer particles as dust. They have been known to segregate materials with in the mix by size as well, with the finer material, collecting beneath the blade e.g., Metal screens can pass fine particles, but retain powder that adheres to the screen materials. Glass containers and laboratory apparatus can adsorb certain materials and may require surface treatment. Plastic containers can retain contaminants, such as animal hairs, while the rest of the sample is transferred with apparent ease. In the other words, validation of a method of analysis, includes, most certainly validation of the method of sample preparation and storage.

Changes in physical characteristics

Loss or gain of moisture during processing can be a problem. Loss can be minimized by keeping samples covered with plastic or aluminum foil. A cold product can be protected from gaining moisture by allowing the sample to come to room temperature before preparation begins. High fat samples such as nuts may be difficult to grind without clogging up the grinder; one technique that is used is to freeze the samples prior to grinding.

Changes in chemical characteristics

When volatile organic constituents are present in any sample, processing may be difficult and needs special care, e.g. maintaining chilled condition to prevent any loss of volatile constituents. Similarly, in case of photo-sensitive chemicals (e.g. natural product pesticides), it is required to process a sample under darkness to prevent degradation on exposure to light.

Portions for sampling

As a general guide, food samples are analyzed in the form they are commonly consumed. Inedible portions, such as stones (e.g. for mango), nutshells, or fish bones are removed and discarded prior to analysis, and suitable note made of how the sample was prepared. The technique used for setting the standard should be used to ensure comparability.

Sampling for Trace metals

Trace metals analysis can present significant problems, For example, the trace metals can be distributed unequally between liquid and solid phases in pickles, canned vegetables and canned fruits. Obviously, such irregular distribution of metals can pose problems for the analyst in establishing the level of metal residues in the product, as well as for those concerned with setting tolerances. Thus, it becomes necessary to analyze both the solid and liquid phases.

Check Your Progress Exercise 4

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

1) Write down some precautions while preparing a sample for analysis?

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11.10 DIFFICULTIES IN SAMPLING

As mentioned earlier, one of the most difficult problem in sampling from a lot, and in subsequent lab subsamples, is trying to obtain a representative sample for the analysis of aflatoxins in raw agricultural commodities. Aflatoxin contamination exhibits a highly erratic distribution, with a reduction in heterogeneity as the food or feed is reduced in particle size. After it was recognized that there was a high rate of variability and within same samples from the same lot, there was a moment towards the collection of larger and larger samples. Sample sizes started, for peanuts with 1 kg, and the size increased as more reliable results were required by food procedures (increasing sample size reduces the number of good lots that are likely to be rejected and the number of bad lots accepted).

11.10.1 Example for Effect of Sampling on Analytical Result

At the present time in the United States, the sample taken from a lot of shelled peanuts of 144 pounds; three 48 pounds samples with portions taken at random from the lot. Examination in the lab is by sequential analysis with first 48 pounds sample ground in a subsampling mill and test portions examined in duplicate. If the average of the test portions is below the established tolerance (set by US Food and Drug Administration), the lot is passed. If the average is above the acceptance level, the lot is rejected. If the findings fall between the two figures the second 48 pound sample is comminuted and the analysis

repeated. If a decision cannot be made to accept or reject the lot, the third 48 pounds sample is prepared, assayed, and the cumulative results considered. The foregoing example point out dramatically the need for attention to lot sampling, lab subsampling, and sample preparation for analysis. While this is a rather extreme case, it illustrates that sampling problems cannot be ignored or treated indifferently. In Canada, while the specified sample sizes are smaller, ranging from 12 to 20 kg. depending on the commodity and the lot size, and minimum number of sampling sites are also stipulated to address the erratic distribution of aflatoxin contamination.

11.11 SAMPLE ACCOUNTABILITY

11.11.1 Documentation

A laboratory sample is generally the starting point for analytical work. The sample may be delivered by mail, courier, flight, or directly by the collector. It may arrive in any of various containers and conditions: frozen, packed in ice, or at room temperature. The package may be sealed or unsealed, and the sample itself may be spoiled or broken. The sample may or may not be accompanied by appropriate documentation to advise the laboratory regarding purpose, test parameters and the conditions of storage, etc.

Once a sample is received, all the circumstances and conditions must be documented as they could have bearing upon the quality or the significance of the test results. It is important for appropriate quality analysis that sample arrives in proper condition with meaningful documents. Procedures for these must be established, continually reviewed, and enforced, to keep poor sample handling and delivery to a minimum level. To avoid any future legal complications, the laboratories are advised to protect themselves with the cautionary statement in the test report indicating that the results relate only to the sample that was tested.

11.11.2 Chain of Custody Form

In most organizations specific sampling procedures are written and the sample collectors are trained regarding their responsibilities. The first important activity is the documentation to ensure product traceability. The sample should be easily identifiable and placed under seal and packing. Shipping and delivery instructions are followed to effect delivery to the laboratory. The documentation consists of a chain-of-custody that accompanies the sample as it moves through the laboratory and subsequent administrative handling. This form is usually prepared in multiple copies for distribution to various units in the organization, may be supplemented with affidavits, dealer's statements, bills or other relevant information that concerns the sample, its origin, the transfers from one custodian to the next and the sample's significance or importance. Information such as sample number, product name and identification, reason for collection, description of the sample and of the method of collection, size of the lot from which the sample was taken, codes, shipment information, collection date, name of the collector, means of transportation, and whether or not sealed are supplied with the sample. If the sample is sealed, the seal includes the sample number, date the seal was affixed, and the collector's signature. The seal is attached to the package in such a way that it must be broken before the sample can be obtained.

11.11.3 Sample Receipt and Handling

The next step in the sample accountability system is receipt of the sample in the laboratory. A dependable record of sample handling is important so that the sample is accepted by a sample custodian who documents the action by completing a sample accountability record. This document should contain the sample number, the name of the product and date received, indicate who received it, describe the method of shipment or delivery, describe the packages received and their condition, and provide space for recording various storage locations before and after analyses. Deliveries of the sample or portions of the sample to the analyst, and its return, will also be recorded on this form. There will be a signed statement concerning the final disposition of the reserve sample. A two-part form can be used for this purpose; one copy remains with the sample custodian and the other moves with the sample through the laboratory and is used by a supervisor for sample management purposes. Some laboratories use a sample receiving log book for sample control. The information entered in the log book is essentially the same as that described for the two-part form.

11.11.4 Monitoring of Samples

The sample accountability in a laboratory can be monitored by a simple computer program; a unique label should be generated and affixed to the sample container, and all the pertinent sample information should be entered into the computer database. The information entered at log–in becomes part of the data base, which is then built up through the manual or automatic addition of sample handling information and analytical data. Worksheet pages or reports can be calculated and printed, and the data base itself latter queried and manipulated for various information and reporting purposes. Regardless of the recording system used, the analytical information generally reported includes a description of the sample, subsampling procedure sample preparation methods used, deviations from methods, validation and recovery experiments (if performed), standards used, source of reference materials, raw data, calculations and description of the reserve sample and how it was prepared for storage after the completion of the analysis. In addition, pertinent supporting documents such as chromatograms, spectra, and other charts are suitably identified with instruments identification, operating conditions, analyst name, sample number and date. If the reserve sample is sealed, the information placed on the seal is shown in the report. The sample is then returned to the sample custodian to be stored for whatever future action may be necessary, or until the sample is destroyed.

11.12 RETENTION OF SAMPLES AND RECORDS

After an analysis is complete and the results reported, the laboratory needs a written policy for guidance on the retention of the samples and the associated records. For samples and records that may be involved in litigation, the storage period can extend for years. For the majority of samples, fortunately, this is not usually the case. The objective should be to destroy samples as soon as it can be analyzed, with certainty that they will no longer be required for further testing or as evidence. The records may be disposed after they are no longer legally or administratively important.

11.12.1 Identify the Properties of Retained Samples

It is very important for the laboratory management to determine whether or not the materials being discarded are hazardous in nature. Although samples themselves may not be hazardous, acid digestions and organic solvent extractions certainly can be hazardous. Sample management includes the proper disposal of samples and laboratory preparations. Standard operating procedures for samples for sample disposal are essential.

11.12.2 Retention Period

Storage periods, obviously, must be determined by each facility depending on its obligations, but clear policy must be in place to prevent both the destruction of important items, and the accumulation of what is essentially junk. From a quality assurance point of view, the improper destruction of active samples or records is low quality performance in violation of policy, and the Quality Assurance (QA) program must provide a means to detect such actions in an effort to prevent their recurrence.

11.13 CASE STUDY

In a 500 T consignment of imported frozen animal carcasses, 300 T labeled as produced by A and 200 T labeled as produced by B is to be checked for residues.

Assumed facts:

i) The carcasses are from an exporter whose products have recently been associated with excessive residues of Permethrin (fat soluble) and Diflubezuron (non-fat soluble).

ii) Carcasses in lot A have trimmable fat, where as those in lot B do not.

iii) The sampling plan is to provide a 95% probability of detection if 10% of the carcasses contain excessive residues.

iv) There is no legal requirement to prepare replicate lab samples.

v) Sampling records are in hard copy form.

vi) Rendering of fat tissue for extraction of lipid acceptable under national law.

Consequent actions and discussions:

i) The consignment is sampled as two separate, suspect lots, A and B

ii) Table 11.3 shows that 29 lab samples should be taken and therefore, as far as practicable, 29 carcasses are selected at random from each lot.

iii) From each selected carcass in lot A, a minimum of 0.5 kg of adhering fat tissue is taken as a (primary) lab sample and a minimum of 0.5 kg of meat (meat does not include bone) is taken as a separate (primary) lab sample.

iv) The carcasses in lot B have no trimmable fat and 29 samples of 2 kg meat are taken.

v) As each lab sample is taken, it is placed in a new polythene bag, securely labeled and sealed, and the sample record completed. The samples are sent to the lab, ensuring that they do not thaw.

Copies of the sample records are given to the owner/custodian of the consignment. Copies are sent with the samples and also retained by the sampling officer.

vi) Fat tissue lab samples from lot a are rendered, the lipid collected and aliquots (analytical portions) analyzed for Permethrin residues. The results are expressed on a whole fat tissue basis.

vii) Bones, if any, are removed from the meat lab samples, which are minced before the determination of Diflubenzuron residues in analytical portions. The results are expressed on the basis of whole meat without bones.

viii) If meat samples from both lots contain Diflubenzuron ≤ 0.05 mg/kg and all samples from lot A contain < 1 mg/ kg Permethrin, lot B is acceptable and lot a is acceptable with respect to Diflubenzuron residues.

ix) If 3 of the 29 fat samples of lot A contain Permethrin > 1 mg/kg, replicate analytical portions of fat from these 3 lab samples are analyzed. Taking into account the analytical uncertainty, if the results conform that the MRL is exceeded, if the 3 carcasses do not comply with the MRL, where as the other 26 do comply with the MRL.

x) If the entire lot is not to be rejected on this basis, lab samples of fat tissue from the remaining carcasses in lot A may be taken for analysis, in order to separate the acceptable carcasses for those that are unacceptable.

11.14 LET US SUM UP

Actions for the management and control of sampling, sample preparation, and sample analysis are summarized below:

1) Work with appropriate persons to develop sampling plans for the various types of products delivered to the laboratory for analysis.

2) Establish sub sampling procedures for various products, giving consideration to the use of composites or individual unit examinations, based on the variability to be expected among sample units and the resources available for their analysis.

3) Prepare guidelines for sample preparation for analysis that will minimize composition change.

4) Choose the appropriate sampling selection method to achieve the intended purpose of taking samples.

5) Describe subsampling within the lab in written procedures which analysts have the responsibility to follow.

6) Manipulation and preparation must be done with care to avoid losses of material.

7) Develop a system to ensure sample accountability.

8) Provide policy for the management of samples when they are no longer needed.

9) Maintain written procedures for sample disposal taking into consideration hazardous waste regulations.

11.15 KEY WORDS

The definitions of sampling terms used in this document are mostly those specified in ISO 7002. Some of the more commonly used terms in acceptance sampling are described in this section.

Lot : A definite quantity of some commodity manufactured or produced under conditions, which are presumed uniform for the purpose of this document.

Consignment : A consignment is a quantity of some commodity delivered at one time. It may consist in either a portion of a lot, either a set of several lots.

Sample (Representative sample) : Set composed of one or several items (or a portion of matter) selected by different means in a population (or in an important quantity of matter). It is intended to provide information on a given characteristic of the studied population (or matter), and to form a basis for a decision concerning the population or the matter or the process, which has produced it.

A representative sample is a sample in which the characteristics of the lot from which it is drawn are maintained. It is in particular the case of a simple random sample where each of the items or increments of the lot has been given the same probability of entering the sample.

Sampling : Procedure used to draw or constitute a sample. Empirical or punctual sampling procedures are sampling procedures, which are not statistical-based procedures that are used to make a decision on the inspected lot.

Total Estimation Error : In the estimation of a parameter, the total estimation error is the difference between the calculated value and the true value of the parameter. The total estimation error is due to:

i) Sampling error

ii) Measurement error

iii) Rounding-of-values or sub-division in to the classes

vi) Bias of the estimator

Sampling Error : Part of the total estimation error due to one or several of the following parameters:

- The heterogeneity of the inspected characteristics.
- The random nature of a sampling.
- The known and acceptable characteristics of the sampling plans.

Acceptable Quality Level (AQL) : The inspection of a lot using either an attributes or variables sampling plan will allow a decision to be made on the quality of the lot.

AQL for a given sampling plan is the rate of the non-conforming items at which a lot will be rejected with a low probability, usually 5%.

Limiting Quality (LQ) : For a given sampling plan is the rate of non-conforming items at which a lot will be accepted with a low probability, usually 10%.

Sampling Plan : A pre-determined procedure for the selection, withdrawal, preservation, transportation and preparation of the portions to be removed from a lot as samples.

11.16 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) The quality of a small sample analyzed is attributed to the lot, which the sample represents. If the sample does not represent the population adequately and efficiently, then the results obtained may not truly represent the quality of the lot.

2) Homogenous population would be uniform and identical at all locations. These populations/samples in which the composition would vary at different locations is heterogenous population.

Check Your Progress Exercise 2

Your answer should include following points:

1) It is very important to arrive at a well designed sampling plan because it is a guide to the people who are going to perform sampling. It also serves as a reminder of the important elements in the over all analytical program.

2) a) Sampling plan is a guide for the whole analytical program.

   b) It is a means for operating on a planned basis which reduces variation.

   c) It serves as a reference document for similar activities in the future.

   d) A document for comparison of performances against objectives.

   e) It also serves as a source for imparting training.

Check Your Progress Exercise 3

Your answer should include following points:

1) i) Probability sampling,

   ii) It is a means for operating on a planned basis which reduces variation,

   iii) Non-Probability sampling,

iv) Bulk sampling,

v) Acceptance sampling,

vi) Sampling by attributes, and

vii) Sampling by variables.

2) Probability sampling is used when a representative sample is desired, and uses principles of statistical sampling and probability, a random selection approach that tends to give each unit an equal chance of being selected.

3) Sampling by attributes:

Advantages: 1. No condition on the mathematical law of distribution of the variable inspected. 2. Greater simplicity of the processing the results on the sample.

Disadvantages: 1. Less effective than variables plans for a same sample size of n increments (Least Quality, LQ is higher). 2. More costly than variables plans because the collected sample requires more increments than those required, for the same efficacy, by a variables plan.

Sampling by variables:

Advantages: 1. More effective than attributes plans for the same sample size of *n* increments (LQ is lower) for the same AQL, they are less expensive than attributes plans because the sample collected requires fewer increments than those required for a same efficacy, by attributes plans.

Disadvantages: They cannot be used in all cases because to validate the calculation formulas of the inspected variable must necessarily or approximately follow a normal law.

4) Set the values of m, M, n and c → Collect the sample with n items → Inspect each item in the sample → Accept the lot if: number of marginally defective items (i.e. a concentration of micro-organisms between m and M) ≤ c. Immediately reject the lot if the concentration of the micro-organisms in any item > M and / or the number of marginally defective items > c.

Where, m: accptable concentration; M: unacceptable concentration; c: maximum allowable number of marginally acceptable sample units; and n is the number of sample units selected randomly from the lot.

5) a) The lots shall be thoroughly inspected before sampling to design a good sampling plan.

b) Suitable sampling devices shall be identified and used while sampling.

c) Compatible containers shall be identified and used. The material of the container shall not cause any undue contamination to the quality of sample collected. For example, Non-sterile containers shall not be used while the sample has to undergo microbiological tests.

d) Suitable packing and delivery method.

e) Provision of appropriate environmental conditions. For example, When there is a need to determine Volatile Organic Constituents in a sample of water, it has to preserved/transported at a temperature 4 to 8°C.

6) Root and bulb vegetable- 5 kg, cereal grains- 2 kg, poultry eggs- 12 eggs, liquid milk- 0.5 L, liquid products- 0.5 L

Check Your Progress Exercise 4

Your answer should include following points:

1) a) Use a suitable method for homogenization. For example, Liquids can be homogenized by stirring, shaking or by blending and take an aliquot. Solids can be homogenized by grinding, pulverizing and volume reduced by coning and quartering.

b) Use clean and suitable sampling devices and containers. For example, A scoop used for sampling a food shall be sterile.

c) A glass container is not compatible for collecting water sample intended for the determination of metals, since metals like sodium are absorbed by glass.

d) Moisture content of sample changes with surrounding temperature. Suitable precautions shall be taken to retain the originality.

e) Maintain suitable environmental conditions to minimize expected chemical changes if any. For example, A food sample for microbiological enumeration shall be collected in a sterile container and be stored/transported in chilled condition.

f) If the sample is presented in both liquid and solid phases, homogenize both phases before a test portion is taken.

11.17 SOME USEFUL BOOKS

Nielsen, S. Suzanne (2003). *Food Analysis Laboratory Manual*, 3rd Edition, CHIPS Publishers, U.K.

International Organization for Standardization (ISO): www.iso.org

Codex Alimentarius (CODEX), www.codexalimentarius.net

Agriculture and Processed Food Products Export Development Authority (APEDA), Ministry of Commerce and Industry: www.apeda.com

Bureau of Indian Standards (BIS), Ministry of Consumer Affairs, Food and Public Distribution: www.bis.org.in / www.fcamin.nic.in

Export Inspection Council of India (EIC), Ministry of Commerce and Industry: www.eicindia.org

Prevention of Food Adulteration Act (1954), 24th Edition, 2003, Ministry of Health and Family welfare: www.mohfw.nic.in

UNIT 12 PHYSICAL AND CHEMICAL ANALYSIS OF FOODS

Structure

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12.5 Let Us Sum Up
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12.8 Answers to Check Your Progress Exercises
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12.0 OBJECTIVES

After reading this unit, we shall be able to:

- explain the physical properties of foods;
- analyze the food for its chemical composition;
- explain the importance of physico-chemical properties of foods;
- define the chemical constants of oils and fats; and
- discuss the advantages and disadvantages of a particular techniques used for food analysis.

12.1 INTRODUCTION

Analysis plays an important role in assessment and maintenance of food quality and safety, both in industry as well as for enforcement authorities at national and international levels. Earlier, food analysis was concerned with food adulteration only. Now-a-days there is an increasing tendency to examine food from a more positive and broader view point. Processed foods are produced within the limits of prescribed manufacturing formulations, set also to comply with legal and/or other requirements. In many food laboratories, most of the routine work is confined to proximate (e.g. moisture, protein, carbohydrate, lipid, fiber, ash) analysis and the analysis of additives and contaminants. This is done by analysis of the product at different stages of processing starting at the farm level. The regulatory requirements for the analysis of food additives and contaminants at very low level have necessitated the development of instrumental techniques suitable for rapid assessment. In case of proximate analysis, the methods may vary for different category of food products. Hence, the results obtained for a particular food constituent depends on the procedure adopted. However as long as the same standard procedure is applied to the same food each time, the results are usually repetitive and thus provide an adequate basis for interpretation.

In food industry, various food components and parameters are analyzed in both raw and processed products. Knowledge of chemical composition of food is important to health, well being and safety of the consumers. Knowledge of the chemical and biochemical composition of food is useful to manufacturers in understanding the importance of various nutritional constituents so that the amount of essential nutrients may be maintained or improved during and after processing. The knowledge of the principles of different food analysis techniques is useful for selection of appropriate technique for analyzing a particular food. The compilation of physical and chemical techniques in this unit would be helpful in understanding the basic principles of food analysis.

12.2 PHYSICAL PROPERTIES

The physical properties of food products have not received adequate attention from the food scientists, although a thorough understanding of the physical changes and principles involved in delivering food products from farm to consumers is essential. Important physical parameters are briefly described below:

12.2.1 Specific Gravity/Density

The specific gravity of a substance is the ratio of density of the material to the density of water at a specified temperature. Specific Gravity can be expressed as

$SG = \rho / \rho H_2O$

where

SG = specific gravity

ρ = density of fluid or substance (kg/m^3)

ρH_2O = density of water (kg/m^3)

It is common to use the density of water at 4 ^{o}C (39°F) as reference - at this point the density of water is at the highest - 1000 kg/m^3 or 62.4 lb/ft^3. Water is the standard for solids and liquids, while hydrogen is the standard for gases.

The density of a liquid at a particular temperature is the mass (e.g. gram) of unit volume (e.g. 1 mL) of the liquid.

$$\rho = \frac{m}{V}$$

The density of solid indicates the weight of a substance held in a unit volume. Densities of liquids are generally measured either by weighing a definite volume of the liquid in a density bottle or pyknometer or by determining the buoyancy acting on a sinker immersed in a liquid. The same principal is used in lactometer for determining density of milk. When sufficient liquid is available, the density can be determined by means of hydrometers or more accurately by means of the Westphal balance.

12.2.2 Specific Heat Capacity

Specific heat capacity of a substance is the amount of heat required to raise the temperature of that substance by one degree centigrade. Specific heat of a substance helps in calculating the amount of heat required to raise the temperature of a substance by a certain amount. Unit of specific heat capacity is J $g^{-1}K^{-1}$ or cal $g^{-1}K^{-1}$

For example, how much heat would be required to raise the temperature of 100 g of water from 30°C to 65°C?

Formula:

Units of heat (Calories) = $mc\Delta T$, where m is the mass in g, c is specific heat capacity and ΔT corresponds to change of temperature

$$= 100 \text{ g} \times 0.94 \times 35°\text{C}$$

$$= 3290 \text{ calories}$$

12.2.3 Surface Tension

Surface tension is defined as the force acting upon a line of unit (1cm) length on the surface of a liquid. Surface tension is a state of stress at the surface of a liquid, which occurs due to inward force of attraction on the surface molecules as result of which the upper surface of a liquid behaves like a stretched membrane. Surface tension is a manifestation of the forces of attraction that hold the molecules together in the liquid (or solid) state; thus liquid droplets tend to become spheres – the form of least surface area – because of the material cohesion of molecules. High surface tension is found with liquids that have strong cohesive forces and consequently high boiling points whereas volatile organic liquids have low surface tension. For a given liquid, the surface tension decreases with rise of temperature and becomes zero at critical point.

Surface tension may be measured by several ways:

A. *Capillary rise method:* If a capillary tube is placed in a liquid it is found that the liquid rises in the tube. To determine the height, to which the liquid

rises in the tube, it is measured by cathetometer or travelling microscope. Surface tension is determined by following equation:

$$\text{Surface tension } (\gamma) = \frac{r \times h \times d \times g}{2}$$

where,

r = radius of capillary tube

d = density of the liquid

h = height to which the liquid rises, and

g = acceleration due to gravity.

B. *Drop-weight method:* This is one of the simplest method. The size of drop issuing from a capillary orifice is governed by the surface tension of the liquid. The instrument employed is called a stalagmometer. The surface tension of a liquid food material like milk can be determined by comparing with water at same temperature. A tube of uniform bore is used and the number of drops falling per unit of time are counted and compared to that of water. Hence, a liquid showing 200 drops as compared to water with 100 drops would have a surface tension one-half as great as that of water.

C. *Torsion balance method:* A more accurate method is the platinum ring procedure. The surface tension can be measured by the force required to detach a horizontal ring of platinum wire from the surface of a liquid. The ring is connected to a delicate balance and the pull required to draw it out of a liquid is measured by a torsion balance.

12.2.4 Viscosity

Viscosity is a property of a liquid closely related to the resistance to flow. It is the frictional effect due to the passage of one layer of fluid (liquid/gas) over another. The coefficient of viscosity is defined as the force required per unit area to maintain unit difference of velocity between two parallel planes in the fluid at 1cm apart. The kinematic viscosity of a liquid is equal to the ratio of the dynamic viscosity and density of the liquid at the same temperature. The unit of kinematic viscosity is stokes (S) and the unit of dynamic viscosity is dynes/cm^2 (Poise). Viscosity of liquid may be determined by any method that will measure the resistance to shear offered by the liquid.

When a liquid flows through a capillary tube of radius r for a time t, under a constant pressure head p, the volume of liquid v issuing from the tube is given by :

$$v = \frac{\pi \times p \times t \times r^4}{8 \times l \times \eta}$$

Where,

η = Coefficient of viscosity, and

l = length of the tube.

It is difficult to determine the absolute coefficient of viscosity for a liquid, however; the relative viscosity of a liquid with respect to water may be determined. The simplest way to do this is to make use of Ostwald viscometer.

The viscosity may be determined by the method of the falling sphere, which depends on the time taken for a sphere to fall through a given distance. This is

used for the measurement of viscous liquids. The viscosity of liquid is calculated by the following equation.

$$\frac{\eta}{\eta_s} = \frac{(D-d) \times t}{(D-d) \times t_s}$$

Where,

η = Coefficient of viscosity of the liquid,

D = density of sphere,

d = density of liquid, and

t = time taken. The subscript s refers to a standard liquid, the viscosity of which is known.

A wide range of viscosity can also be measured by Brookfield viscometer using different spindle and speed. Brookfield viscometer is usually suitable for the measurement of highly viscous liquids.

12.2.5 Refractive Index

The measurement of refractive index of certain substances is helpful in identifying and establishing their purity, in determining the molecular structure of organic compounds and quantitative analysis of certain types of solutions. The Abbe refractometer is particularly used in food analysis and in the testing of oils. It covers a wide range of refractive indices and uses a very small amount of sample. In making a refractive index measurement, the beam of light is usually passed from air through the solid or liquid medium being measured and then through a glass prism and out into the air again. The refractive index may be calculated from the angle through which a telescope must be turned in order to pick up the emerging beam on a cross hair. The angle measured includes the refraction at the liquid-glass interface and at the glass-air interface.

12.2.6 Filth

Any foreign matter in product associated with objectionable conditions or practices in production, storage, or distribution included are filth, decomposed material and miscellaneous matter *viz.*, sand, soil, glass or other foreign substances excluding bacterial counts.

A. *Filth:* Any objectionable matter contributed by animal contamination of product such as rodent, insect, or bird matter; or any other objectionable matter contributed by unsanitary conditions.

B. *Heavy filth:* Heavier filth material is separated from food products by sedimentation techniques based on different densities of filth, food particles and immersion liquids such as chloroform. e.g., insect and rodent excreta pellets and pellets fragments, sand and soil. When chloroform is mixed vigorously with the plant material and allowed to separate, most of the plant tissues rise and the heavy extraneous matter settles to the bottom, which can be separated by draining on filter cloth.

C. *Light filth:* Lighter filth particles that are oleophillic and are separated from product by floating them in an oil-aqueous liquid mixture e.g., insect fragments, whole insects, rodent hairs and feather barbules. It is difficult to wet all the insect parts without creating frothy emulsion of the plant

material. Droplets of oil adhering to sides of trap flask may prevent insects from rising. To overcome it, the oil is worked thoroughly into the water-food mixture without incorporating air. If an emulsion is formed, capryl alcohol or ethanol may be used to break the emulsion.

D. *Sieved filth:* Filth particles of specific size ranges are separated quantitatively from the product by use of selected sieve mesh sizes.

12.2.7 Particle Size

Particle size analysis means the separation of a sample of material into fractions of different average diameters. Hand sieving is the most common method. The sieves are arranged in a nest, the outer casing of each sieve fitting the casing of the sieve below it and the nest provided with a top cover and bottom blank pan. The weighed sample is placed on the coarsest sieve and the whole nest is given a preliminary shaking after which each sieve is shaken separately to complete the separation. Both time and intensity of shaking must be kept as uniform as possible. A mechanical shaker is preferable to hand shaking since time and intensity of shaking can be exactly repeated from sample to sample. For separation finer than 200 mesh elutriation is used. Elutriation is the separation of material by the action of a rising current of fluid. Microscopic sizing analysis is a form of direct counting and is theoretically more accurate than either screening or elutriation. However, the method is only a guide, since the quantity of material visible in the microscopic field is very minute.

Check Your Progress Exercise 1

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

1) What is filth? Briefly discuss the separation technique for the heavy filth?

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2) Correlate heat and specific heat?

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3) How can you measure the viscosity of liquid food product with low consistency?

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4) Differentiate the specific gravity and density of foods?

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5) Define surface tension? Suggest a simple method for its determination?

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12.3 CHEMICAL PROPERTIES

12.3.1 Moisture

Water, the simplest of all constituents of foods, is one of great concern to producer, consumer and chemist. The weight of food has little significance unless taken into consideration with the water content. The accurate determination of moisture poses many challenges. One of the problems is the difficulty of separating all of the water from the food sample, resulting in underestimation of moisture content. On the other hand, harsher conditions to remove all moisture from a food may simultaneously cause decomposition of the product, which may result in the production of water along with/or a loss in sample mass. Thus the accuracy of the method would be severely in question. Most of the methods for the estimation of water in foods depend on the loss in weight on heating. An exposure to the air of the drying oven causes the oxidation of certain oils and other constituents; a gain in weight of such constituents offsets the loss in weight due to moisture. To obviate the error, the drying should be performed in vacuum. The loss in weight on heating is not entirely because of water as other volatile substances evident to the sense of smell are present in most foods, although the amount is too small. Significant loss of volatile compounds from the food is another potential difficulty in the determination. Most of the spices however contain notable quantities of volatile oil that pass off with the water.

Analytical methods of moisture determination can be classified in two ways.

1) **Direct methods:** Moisture analysis normally involves removing water from the food samples by drying, distillation, extraction and its quantity is measured by weighing, titration and so forth, e.g., oven drying, vacuum drying, freeze drying, distillation method, Karl Fischer method, chemical desiccation, thermo-gravimetric analysis and gas chromatography.

2) **Indirect methods:** The indirect methods must be calibrated against standard moisture values that have been precisely determined using direct methods, e.g., refractometry, infrared absorption, near infrared reflectance spectroscopy, microwave absorption, dielectric capacitance, mass spectrometry, NMR spectroscopy, neutron scattering method, etc.

I) Air-Oven Drying Method

It is one of the most common and widely used methods for routine moisture determination. The ovens should be thermally regulated to ±0.5°C and have minimal temperature variations (<±3°C) within the oven. The main criterion of food for moisture determination by air-oven drying is that sample should be thermally stable and should not contain significant amount of volatile compounds.

II) Vacuum Oven Drying Method

It is the standard and most accurate drying method for moisture analysis of foods. The AOAC methods generally recommend that moisture content of food can be determined by heating foods at 98 to 102°C at a pressure of 25-100 mm Hg for 2-6 h. Lower temperatures (60-70°C) can be used for heat sensitive/ sugary food products in sugar to prevent decomposition used for products like jam, confectionery etc.

III) Distillation Method

Two types of distillation procedures exist for moisture determination; (a) Direct distillation and (b) Reflux distillation.

a) Direct Distillation

In this method, a food is heated in a liquid immiscible in water and has a high boiling point (e.g., mineral oil). The water in the food like spices and herbs distils directly from this liquid, condenses and collects in a graduated tubes; the volume of the water removed is then measured e.g., Spices, herbs.

b) Reflux Distillation

It makes use of the azeotropic properties of solvent mixtures. During heating, water and an immiscible solvent (toluene or xylene) distil off together at a constant ratio and frequently at a temperature lower than the boiling point of either component. For example, the boiling points of water and toluene are 100°C and 110.6°C, respectively, but the boiling point of the binary mixture is 85°C; the distillation ratio of the mixture is 20% water and 80% toluene. As water is denser than toluene, the water is again collected in a suitable measuring apparatus where it separates and has its volume measured. A representation of the reflux distillation apparatus is shown in Fig. 12.1.

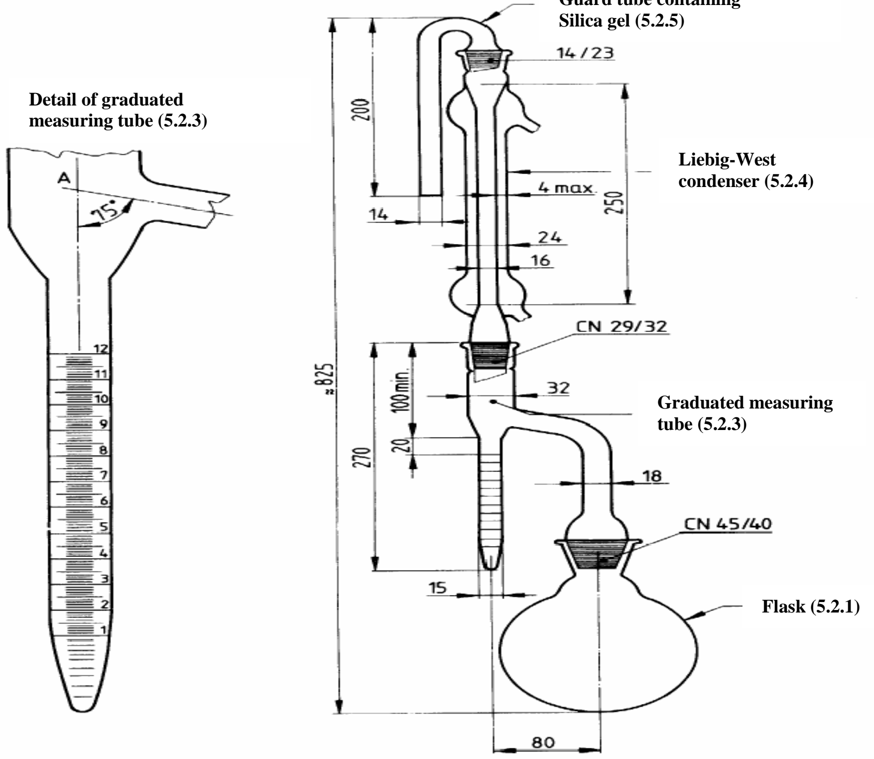

Fig. 12.1: Dean and Stark Apparatus for Moisture Determination

IV) Karl Fischer Titration Method

The Karl Fishcer (KF) titration has become a standard method for the moisture analysis in liquids and solids due to its selectivity, high precision and speed. The method is particularly suitable for food where heating methods give erratic results. This method has been approved for dried vegetables, oils and fats, cocoa products and liquid molasses. The KF titrimetric method is based upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine dissolved in pyridine and an alcohol. The Karl Fischer reagent consists of iodine, pyridine, SO_2 and methanol. The titration is conducted either by volumetric method (where the end point is brown colour, determined visually) or by coulometric titration, where the end point is determined by a potentiometer. Food samples may be directly introduced into the reaction vessel if the water is easily accessible to the reagent. In solid foods where water is not accessible, the water is frequently extracted into anhydrous methanol and then estimated.

V) Refractometry

The refractive index is a ratio of the sine of the angle of incidence made by a ray in air to the sine of the angle of refraction made by the ray in the material being tested. The refractive index increases as the moisture content decreases. By measuring the refractive index of a solution, the moisture content can be rapidly determined using an appropriate calibration curve. Refractometry is best suited for high sugar products *viz.*, fruit products, syrups and honey. For solid or semi-solid foods, the sample can be homogenized with an anhydrous solvent (e.g., isopropanol) and then the refractive index of the solution can be

measured using a refractometer. The moisture content of the sample may be calculated using the calibration curve (produced by measuring refractive index of solutions containing the same solvents with known amounts of added water) and the mass of food homogenized in the solvent.

12.3.2 Water Activity

The water activity (a_w) of a food describes the energy status of the water in the food. It is the ratio of vapour pressure of water in a sample to saturation vapour pressure at sample temperature. The water activity is not influenced by the total quantity of water in a sample but only by that fraction which is least tightly bound. Temperature affects a_w due to changes in water binding, solubility of solution in water and the state of sample matrix. Most high moisture foods exhibit negligible change with temperature. The water activity controls all aspects of microbial growth by lengthening the lag phase of microbial growth. The water activity influences non-enzymatic browning, lipid oxidation, degradation of vitamins, enzymatic reactions, protein denaturation, starch gelatinization and starch retrogradation. The water activity is usually measured by using different hygrometers *viz.*, hair hygrometer, resistance sensor, capacitance sensor, dew point hygrometer, etc.

12.3.3 Protein

All natural foods contain protein, although trace amounts are found in honey and maple sugar. The quantification of total protein in food and food products can be performed directly or by determining total nitrogen from conversion of crude protein using a suitable conversion factor. The protein content is calculated from the total nitrogen determined by either Kjeldahl method or Dumas/Pregl-Dimas method. Amides (abundant in young shoots), ammonium salts, nitrates, lecithin, nucleic acid, purines of tea, coffee, cocoa and meat extracts in addition to protein contain nitrogen in varying proportions. Although small, these compounds thus add error to the calculated protein estimate. However, the protein calculated by factor is a valuable figure, not only because it represents approximately the true protein present but also because it is an index of the content of other groups. The protein content can also be determined directly by formal titration, UV spectrophotometry, Lowry method, Dye binding method, IR spectrophotometry, NMR spectroscopy, turbidimetry, refractometry, etc.

I) Direct Method

Since foods contain mixtures of proteins, the methods for the direct determination of proteins need to be calibrated against a reference standard for nitrogen, e.g. Kjeldahl method.

i) Formal Titration Method

When formaldehyde is added to neutralized aqueous solution containing protein, the $-NH_2$ group of protein converts to methylene-amino group ($-N=CH_2-$) with the release of proton. This may be titrated.

ii) Spectrophotometric Method

The Lowry method is based on the amplification of the biuret reaction (complex of cupric ions with protein) by subsequent reduction of the Folin phenol reagent (mixed acids of phosphomolybdic and phosphotungstic) by

tyrosine and tryptophan. This redox reaction is accompanied by the formation of a blue colour (λ_{abs} 745 – 750 nm), which is highly pH dependant (10-10.5).

II) Indirect Method

i) Kjeldahl Method

It has wide acceptance for the determination of protein in food products. The method follows three steps:

Digestion – Decomposition of organic matter by heating in the presence of concentrated sulphuric acid, the end product is ammonium sulphate solution.

Distillation – Ammonium sulphate is converted into gaseous ammonia by addition of an excess base, followed by boiling and condensation of the ammonia in a receiving solution (acid).

Titration – Quantification of the unreacted acid in the collecting vessel.

The rate of digestion and the completeness of the breakdown of nitrogenous compounds to ammonium sulphate mainly depends upon the heat input, amount of boiling point elevator of acid (alkali sulphate), addition of catalyst (mercury, cupper sulphate, titanium dioxide), oxidant (hydrogen peroxide), reflux rate of sulphuric acid and length of digestion.

Ammonia is liberated from the acid digestion mixture by distillation in the presence of alkali (50% NaOH). A total recovery of ammonia from the digest can be obtained within 5 to 20 min by direct distillation and about 10 min by steam distillation.

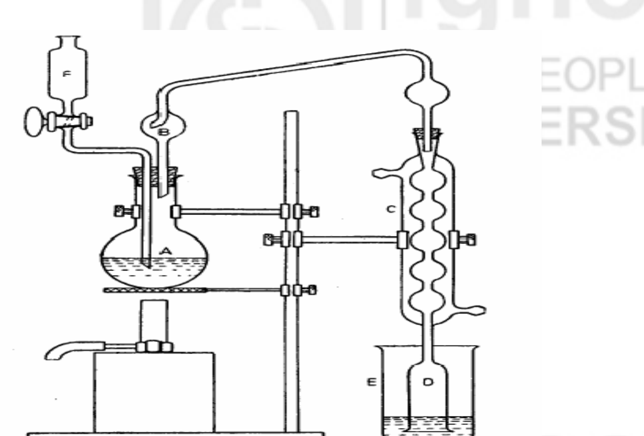

Fig. 12.2: Kjeldahl Nitrogen distillation assembly

ii) Dumas Combustion Method

The protein content of foods can be estimated by the determination of elemental nitrogen using instruments based on the Dumas principle. In these instruments, the nitrogen containing constituents of the sample are combusted at high temperature about 1000°C in the presence of oxygen to oxides of nitrogen (NOx) and then reduced over copper or tungsten to gaseous nitrogen which is measured by gas solid chromatograph using thermal conductivity detector. This method offers significant advantage over Kjeldahl method i.e., shorter analysis time (3-4 min), but these instruments appear to have limited usefulness for some food products because they can only deal with very less amount of sample.

12.3.4 Fat

The oils and fats from oilseeds and fruits as well as from animal fatty tissues correspond quite closely with those extracted by diethyl ether. Practically, all the sterols and phosphorus containing organic compounds notably the lecithins are extracted with the glycerides. Essential oil and resins are the chief constituents of the ether extract of certain spices. Similarly, pepper contains nitrogenous ether soluble substance, piperine (alkaloids). Other solvents *viz.*, chloroform, carbon tetrachloride, carbon disulphide and petroleum distillates of lower or higher boiling points dissolve fats and oils and can be used but the yield and composition of the extract differ somewhat with the solvent. Free fat can be extracted by the less polar solvents such as petroleum ether and diethyl ether, whereas the bound fat requires more polar solvents *viz.*, alcohols for their extraction. The bound fat may be broken down by hydrolysis or other chemical treatment to yield free fat. Hence, the amount of extracted fat found in food products will depend on the method of analysis used.

I) Direct Solvent Extraction Method

The free fat content can be conveniently determined in foods by extracting the dried and ground material with petroleum ether or diethyl ether in Soxhlet extraction apparatus (Fig. 12.3). Extraction in the presence of alcohols causes the release of lipoidal substances bound to proteins and carbohydrates *viz.*, phospholipids and glycolipids. Hence, maximum extraction is obtained by a mixture of polar and non-polar solvents. This procedure co-extracts water and water soluble substances. Hence, the residue after solvent removal and the addition of anhydrous sodium sulphate needs to be extracted with petroleum ether.

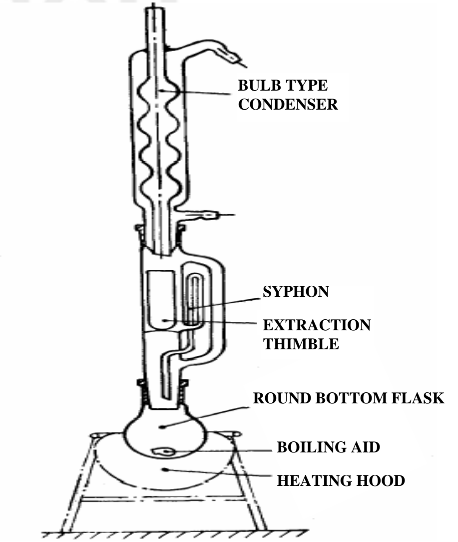

Fig. 12.3: Soxhlet extraction apparatus

II) Solubilization Extraction Method

Bound fat can be made free if the food sample is dissolved completely prior to extraction with polar solvents. Dissolution of the food can be achieved by acid or alkaline hydrolysis.

In acid hydrolysis method, the sample is heated on a steam bath with dilute HCl and boiled for 30 min. The sample solution is filtered through a wet filter paper and washed with hot water. The filter paper is then oven dried and placed directly into a Soxhlet apparatus and extracted with ethyl or petroleum ether or dichloromethane.

In alkali hydrolysis method (Rose Gottlieb method), the material is treated with ammonia and alcohol in cold and the fat is extracted with diethyl ether-petroleum ether mixture. The alcohol precipitates the protein, which dissolves in the ammonia; the fat can then be extracted with ether. Petroleum ether is then added as it reduces the proportion of water and hence all non-fatty substances.

(All dimensions in millimeters)

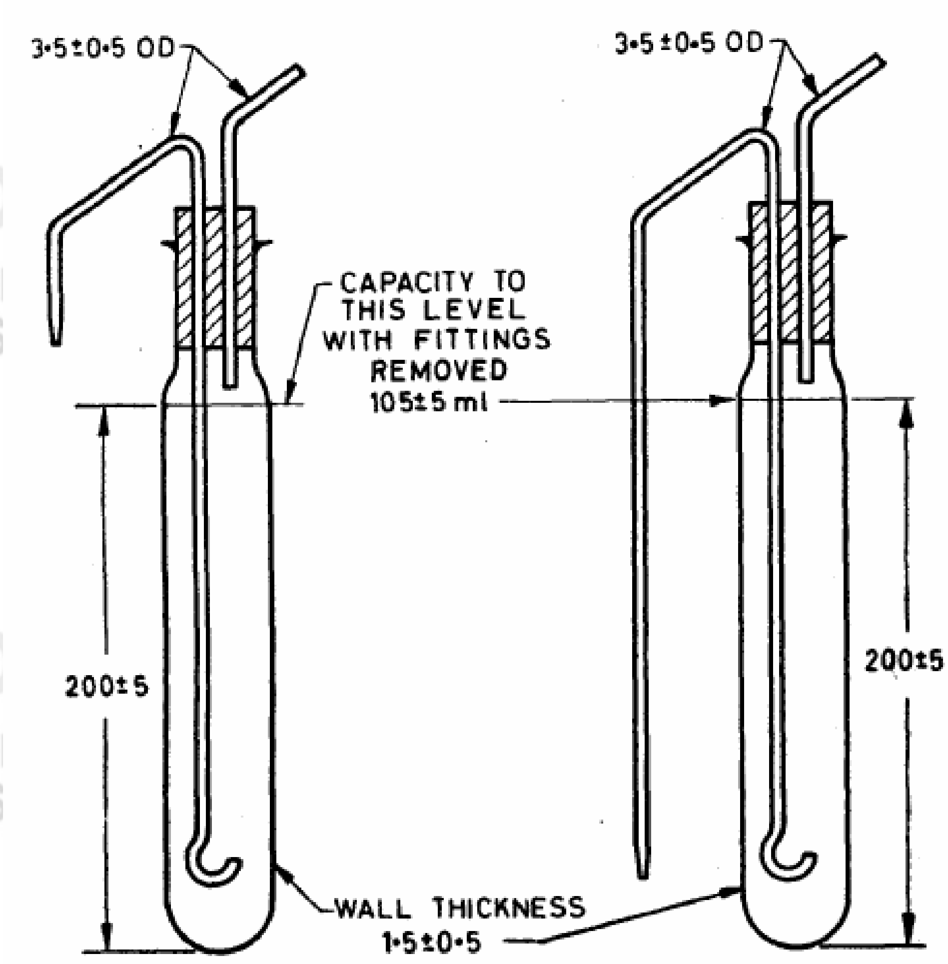

Fig. 12.4: Fat extraction Rose Gottlieb tubes with fitting

III) Volumetric Method

These involve dissolving the sample in sulphuric acid and centrifuging out the fat in specially calibrated glass vessels (butyrometers). The Gerber method is commonly employed for the routine determination of fat in milk and dairy products.

12.3.5 Volatile Oil

The method involves distilling the volatile oil over with boiling water, condensing and collecting the oil in a measured volume of xylene in a graduated tube. (Fig. 12.5).

ELEVATION

All Dimension in millimeters.

Fig. 12.5: Apparatus for determination of volatile oil

12.3.6 Crude Fiber

The crude fibre representing the cell wall material left after boiling with dilute acid and alkali in the process, is a mixture of cellulose, lignin and pentosans, together with sand, silica and other mineral matter locked in the tissues and little nitrogenous matter after grinding and defatting, boiling with sulphuric acid solution, and separation and washing of the insoluble residue. This residue is boiled with sodium hydroxide solution, separated, washed, and dried and the insoluble residue is then weighed. The loss in mass on incineration is also noted.

12.3.7 Dietary Fiber

The current surge of interest in dietary fibre is attributed to its prophylactic and curative properties against colon cancer, coronary heart disease, obesity, gallstones, constipation, bowel irregularities and even hemorrhoids. The food industry is responding to the desires of today's consumers for fibre-rich products that can be used to foster their health, vitality and well-being. Dietary fiber refers to a macro-constituent of food, which includes the remnants of edible plant cells including polysaccharides (cellulose, hemicellulose, gums, mucilage, pectin), lignin, and associated substances *viz.*, oligosaccharides, waxes, cutin and suberin that are resistant to digestion in the alimentary tract of human being. Dietary fiber is separated from other constituents of food by means of enzymatic hydrolysis while crude fiber is separated by means of acid and alkali hydrolysis of other food constituents.

I) Total Dietary Fibre (TDF)

Duplicate test portions of dried foods (fat extracted if containing >10% at) are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol are added to precipitate soluble dietary fibre. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue is weighed. One duplicate is analyzed for protein and other is incinerated at 525°C and ash is determined.

$$\text{Total dietary fibre (\%)} = \frac{\text{Weight of residue} - \text{weight (protein + ash)}}{\text{Weight of sample}} \times 100$$

II) Soluble Dietary Fibre (SDF)

Duplicate test portions of dried foods, (fat extracted if containing >10%fat), are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Insoluble dietary fibre is removed by filtering and washing residue with water. Soluble dietary fibre in filtrate is precipitated by adding 95% ethyl alcohol to filtrate. Precipitate is filtered and washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone, dried and weighed. One duplicate is analyzed for protein and second is incinerated at 525°C to determine ash. Soluble dietary fibre = Weight residue – weight (protein + ash).

III) Insoluble Dietary Fibre (IDF)

Duplicate test portions of dried foods, (fat extracted if containing >10%fat), are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Enzyme digest is filtered and residue is washed with warm water, dried and weighed. Insoluble dietary fibre residue value is corrected for protein, ash and blank.

12.3.8 Total Ash

Ash refers to the inorganic residue remaining after total incineration of organic matter. The ash content is an indicator of product quality and the nutritional value of food products. When a high ash figure suggests the presence of an inorganic adulterant, it is advisable to determine the acid insoluble ash.

I) Dry Ashing

Dry ashing is the most standard method for determining the ash content of a food sample. The sample is commonly ignited at 550-600°C to oxidize all organic materials without flaming. The inorganic residue that does not volatilize at that temperature is called ash. The ash content is determined from the loss of weight, which occurs from complete oxidation of sample.

II) Wet Ashing

Wet ashing is usually used for the elemental analysis. Wet ashing commonly employs concentrated nitric acid and perchloric acid or nitric acid and sulphuric acid to oxidize the organic matter of the food sample. These acids are partially removed by volatilization and the soluble minerals remain dissolved in nitric acid. Any silica present is dehydrated and made insoluble. However, great care must be taken when using perchloric acid, because it can be explosive on contact with water.

12.3.9 Acid Insoluble Ash

The acid insoluble ash is a measure of the sandy matter and maxima are prescribed for herbs and spices. Acid insoluble ash is determined by dissolving ash in dilute hydrochloric acid (10% w/w), the liquid filtered through an ashless filter paper and thoroughly washed with hot water. The filter paper is then ignited in the original dish, cooled and weighed.

12.3.10 Sulphated Ash

This involves moistening the ash with concentrated sulphuric acid and igniting gently to constant weight. The sulphated ash gives a more reliable ash figure for sample containing varying amount of volatile inorganic substances that may be lost at the ignition temperature used.

12.3.11 Reducing and Non-Reducing Sugars

I) Lane and Eynon Volumetric Method

Reducing sugar and non-reducing sugar (after inversion) reduces the copper in Fehling's solution to the red precipitate cuprous oxide. The sugar content in a food sample is estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of Fehling's solution. The Fehling's solution is an alkaline solution of copper sulphate ($CuSO_4$. $5H_2O$, 6.9%) and Rochelle salt Sodium Potassium Tartrate ($KNaC_4H_4O_6.4H_2O$, 34.6%). In this method, methylene blue is used as an oxidation-reduction indicator of the end point. The methylene blue is added to the reaction mixture of sugar and Fehling's solution. Its use is based on the fact that it is reduced and completely decolourized by minute amounts of reducing sugar or invert sugar but not so long as any cupric salt is present. The reduction is carried out in a flask in which the liquid is kept boiling constantly to prevent reoxidation. In acid-base indicators, the change is often in the nature of colour i.e. in its position in the spectrum; but with oxidation-reduction indicators it is a change in intensity of colour.

II) Colorimetric Method

Food sample is clarified with the help of suitable clarifying agent and the filtrate has to be free from protein and fat. Phenol solution and concentrated sulphuric acid are added to an aliquot portion of the filtrate, thus producing a colour which is proportional to the amount of carbohydrate present, which is measured photometrically at a wavelength of 490 nm.

12.3.12 Starch

After the sugars present in the sample are leached out, starch is hydrolyzed using acid or enzyme and then the sugar is estimated.

I) Acid Hydrolysis Method

The sugar is leached out by precipitating starch with alcohol and the starch is hydrolyzed with conc. hydrochloric acid and the resulting reducing sugar is determined by titrimetric or by colorimetric method.

Starch (%) = % Reducing sugar × 0.90

II) Enzymatic Hydrolysis Method

Sugars are removed by leaching with alcohol. If much fat and proteinaceous materials are present, the sample is treated with hot ethanolic KOH and washed

with 80% ethanol. The starch in the residue is gelatinized and incubated with amyloglucosidase enzyme at pH 4.5 which converts the starch to glucose which is measured enzymatically using glucoxidase.

Let us answer a few questions before we move to the next section of physical and chemical testing of Fats and Carbohydrates. Microscopic field is very minute.

Check Your Progress Exercise 2

Note: a) Use the space below for your answers.

b) Check your answers with those given at the end of the unit.

1) What are the different techniques used for moisture determination in food products?

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2) Describe the principle of Kjeldahl method used for protein determination?

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3) Differentiate water content and water activity of food?

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4) Differentiate crude fibre and dietary fibre?

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5) What is the significance of ash content in foods?

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6) Explain the role of methylene blue indicator in the determination of sugar?

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12.4 PHYSICAL AND CHEMICAL PROPERTIES OF OILS AND FATS

Modern advances in oils and fats technology and the nutrition science have led to the need for greater awareness of the composition and structure of dietary lipids and many new advanced test methods and analytical procedures have been introduced. This section describes the general methods used to examine oils and fats for their physical and chemical properties and methods for assessing their quality criteria.

12.4.1 Acid Value and Free Fatty Acids

Acid value is the amount of KOH in milligram, required to neutralize the free fatty acids present in 1 g of the oil or fat. It is determined by directly titrating the material in an alcoholic medium with aqueous sodium or potassium hydroxide solution. Free fatty acid is calculated as oleic, lauric, ricinoleic or palmitic acids. Acid value when expressed, as mg of KOH/g of fat should be not more than 4.0 in virgin oils and 0.6 in non-virgin oils.

$$\text{Free fatty acid (\%) (as oleic acid)} = \frac{\text{Titre volume} \times \text{normality of NaOH} \times 28.2}{\text{Weight of sample}}$$

Acid Value = % FFA × 1.99

12.4.2 Unsaponifiable Matter

Unsaponifiable matter is that fraction of oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents. The material is completely saponified with alcoholic potassium hydroxide solution and extracted with petroleum ether. The petroleum ether extract is washed with aqueous alcohol and then again with water. The washed ether extract is evaporated and the residue weighed. Unsaponifiable matter is this residue minus the fatty acid present in it, which is determined by titration with sodium hydroxide solution in alcoholic medium.

12.4.3 Melting Point

Oils and fats are chiefly mixtures of glycerides. They do not exhibit either a definite or a sharp melting point. Therefore, the term melting point does not imply the same characteristics that it does with pure crystalline substances. Fats pass through a stage of gradual softening before they become completely liquid. The melting point is, therefore, defined by the specific conditions of the method by which it is determined. The melting point is determined by taking the solid fat inside a small capillary tube and sample may be compared by measuring the temperature at which under specified conditions a column of fat fixed length rises in an open capillary tube under a definite pressure (slip point).

12.4.4 Solid-liquid Ratio

This provides information on the extent of saturation of triglycerides in fat, e.g. the extent of hydrogenation of oil or the suitability of fat for a particular use. The ratio can be measured by dilatometry. This is based on the measurement of isothermal expansion of the fat. The sample is melted, put in an enclosed calibrated glass tube known as a dilatometer and solidified under standardized conditions. The temperature of the solidified fat is then raised in 5°C stages and the volume of the fat measured each time until it is almost completely molten. By plotting the change in volume against temperature a melting dilation graph is obtained. However, determination of solid-liquid ratio in fat by Nuclear Magnetic Resonance (NMR) method is more suitable and accurate.

12.4.5 Specific Gravity

The specific gravity may be determined with a specific gravity bottle or pyknometer. The temperatures at which the specific gravity is determined shall be reported, namely, sp gr 30°C/30°C or sp gr 95°C/30°C.

12.4.6 Titre Value

When the molten fatty acids are cooled and begin to solidify, the latent heat of fusion is liberated and consequently a sudden rise in temperature can be observed. The sample is prepared from the fat by saponification and subsequent liberation of the fatty acids by dilute mineral acid. The washed and dried fatty acids are transferred to a test tube of specified dimensions in which they are cooled and agitated with a stirrer until solidification becomes noticeable. The highest/simplify temperature recorded, after mixing has been discontinued, is known as the titre value.

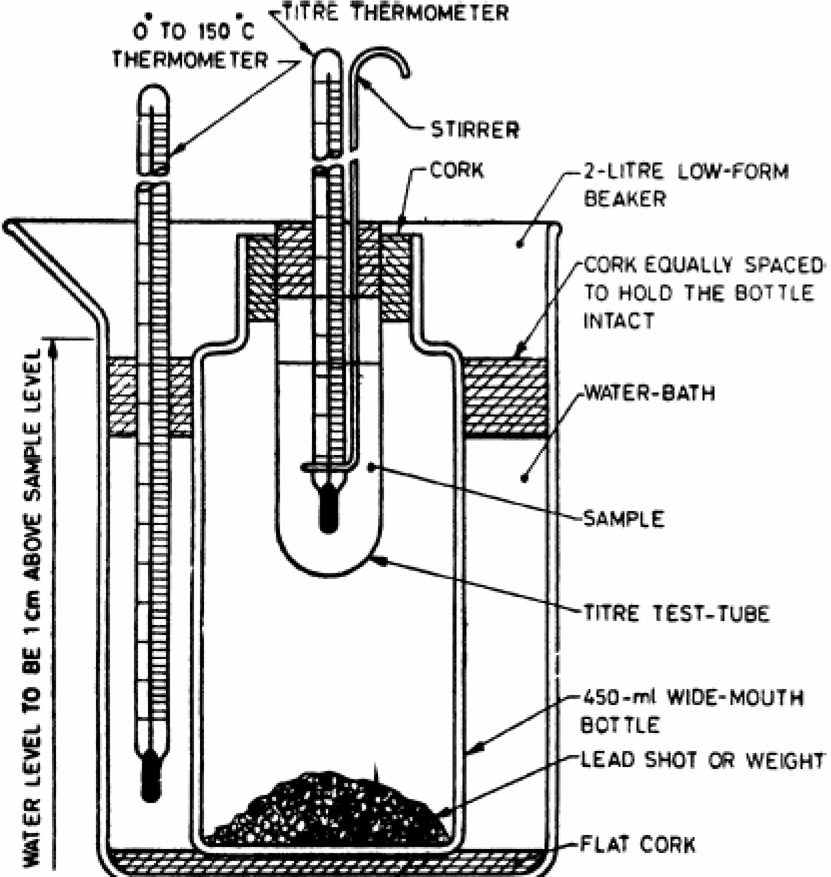

Fig. 12.6: Assembly of Apparatus for Titre Test

Determination of Titre — Fill the low-form beaker with water up to two-thirds of its capacity. Adjust the temperature of water between 15°C and 20°C below the expected titre point when it is not above 35°C, and at 20 ± 1°C when it is 35°C or higher. Fill the test-tube up to the mark with the fatty acid preparation at a temperature 10 to 12°C higher than the expected titre point. Insert the titre thermometer in the centre of the sample and adjust its height so that its immersion mark coincides with the top surface of the fatty acid layer. When the temperature of the fatty acid comes down to about 10°C higher than the titre point, set the stirrer moving in a vertical direction at a rate of about 60 complete up and down motions per minute. The temperature of the fatty acid gradually comes down and stirring is continued until the temperature remains constant for 30 seconds. The stirring is stopped when the temperature begins to rise and the stirrer is raised out of the sample. The highest temperature recorded by the thermometer during this rise is the titre point. Duplicate determinations should agree within 0.2°C.

12.4.7 Colour

This method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond tintometer. The colour may also be measured in a spectrophotometer using carbon tetrachloride as blank at the wavelength of maximum absorption.

12.4.8 Iodine Value (Wij's)

Iodine value is a measure of level of unsaturation in fat. Iodine value is the amount of iodine in (g) absorbed per 100 g of the oil or fat. The material is treated, in carbon tetrachloride medium, with a known excess of iodine monochloride solution in glacial acetic acid (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with standard sodium thiosulphate solution.

$$\text{Iodine value (mg/100g)} = \frac{\text{Titre volume (blank – sample)} \times \text{normality of } Na_2S_2O_3 \times 12.69}{\text{Weight of sample}}$$

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                     I   Cl
                     |    |
- CH=CH-  + ICl → - H-C -C-H-
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$H_2O + ICl \rightarrow IOH + HCl$

$KI + HOH \rightarrow KOH + HI$

$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$

12.4.9 Saponification Value

When fat is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution, the triglycerides hydrolyze, while glycerol and soap are formed. The alkali consumed for this hydrolysis is a measure of the saponification value, which is determined by titrating the excess alkali with standard hydrochloric acid. Saponification value is defined as the amount of KOH in mg required in saponifying completely 1 g of oil or fat. It is also a measure of the mean molecular weight of the fatty acids originally bound as triglycerides.

$$\text{Saponification value} = \frac{\text{titre value (blank – sample)} \times \text{normality of HCL} \times 56.1}{\text{Weight of sample}}$$

12.4.10 Acetyl Value and Hydroxyl Value

The acetyl value of fats or oils defined as the amount of KOH in mg required for the neutralization of the acetic acid obtained by the saponification of 1 g of the acetylated product.

The process consists in acetylating the oil or fat with a measured quantity of acetic anhydride in pyridine decomposing the excess anhydride by boiling with water and then, after the addition of sufficient butyl alcohol to give a homogeneous solution, titrating with alcoholic alkali. A control test with the acetic anhydride and pyridine without the oil or fat provides a measure of the acetic anhydride available for acetylation; a similar test with the oil or fat and the pyridine without the acetic anhydride provides a measure of the free fatty acid present. From the figures obtained, the acetyl value or the hydroxyl value of the fat is calculated.

12.4.11 Reichert-Meissl (RM) Value

Reichert Meissl value is a measure of water soluble steam volatile fatty acids chiefly butyric and caproic acids. RM value is the number of ml of 0.1N NaOH solution required to neutralize the steam volatile water soluble fatty acids distilled from 5 g of oil or fat under the precise conditions. The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulphuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution. RM value is a unique test used for evaluation of adulteration of milk fat with other fat.

12.4.12 Polenske Value

Polenske value differs from the RM value in that it is a measure of 'steam volatile' but of water insoluble fatty acid like caprylic, capric and lauric acids present in oils and fats. The condenser, the 25-ml cylinder and the receiver used in the Reichert-Meissl value determination are washed into the filter paper through which the distillate was filtered for that determination. After rinsing, the residue on the filter paper is taken up with ethyl alcohol and titrated with standard sodium hydroxide solution.

12.4.13 Rancidity

The reactions that take place when a fat becomes rancid are only partly understood. It appears that the more common type of rancidity that results in the formation of a rancid odour and taste is due to oxidation at the double bonds. Apparently, peroxides are formed in the early stages of the process and these are later decomposed into aldehydes, ketones and acid fragments. The evaluation of rancidity can be done by determining different oxidation products.

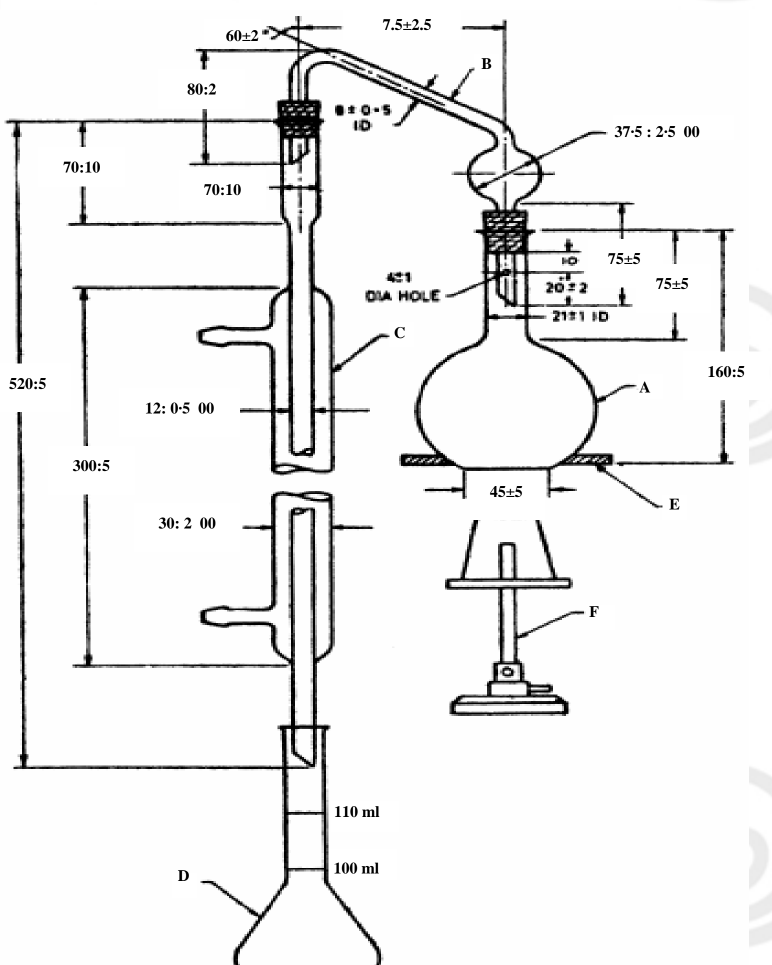
I) **Kreis Test:** The method employs phloroglucinol as a reagent in ether in the presence of concentrated hydrochloric acid. If epihydrinaldehyde is present, a reddish addition product is formed, indicating rancidity. Addition reaction of aldehydes with phloroglucinol can be determined using spectrophotometric detection at 540 nm.

II) Peroxide Value: The peroxide value is an indicator of oxidative rancidity in fats. However, the incipient stages of rancidity can be detected by this test before the spoilage can be detected organoleptically. The peroxide value is a measure of the peroxides contained in a sample of fat, expressed as milli-equivalents of peroxide oxygen per kg of the material. The material in an acetic acid-chloroform medium is treated with an aqueous solution of potassium iodide. The liberated iodine is titrated with standard sodium thiosulphate solution. The peroxide value of fresh oil is less than 10.

$$\text{Peroxide value (meq/1000g)} = \frac{\text{Normality of } Na_2S_2O_3 \times \text{titre volume} \times 1000}{\text{Weight of sample}}$$

$$ROO + KI \xrightarrow{H^+/\Delta} ROH + KOH + I_2$$

$$2Na_2S_2O_3 + I_2 + \text{starch} \rightarrow \text{starch} - I_2 + Na_2S_4O_6 + 2NaI$$



All dimensions in millimeters.

Fig. 12.7: Reichert-Meissl Distillation Apparatus

Check Your Progress Exercise 3

Note: a) Use the space below for your answers.

b) Check your answers with those given at the end of the unit.

1) What are the physical parameters used for the quality evaluation of oils and fats?

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2) Define the iodine value? What is the significance of iodine value?

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3) Define RM value? Briefly discuss its method of determination?

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4) Which test will you perform to evaluate the rancidity in oil?

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12.5 LET US SUM UP

The physical and chemical analysis techniques used for food analysis are enumerated in this unit. The principles and methods of determining different physical parameters of food products are discussed in this unit. The chemical analysis of foods includes proximate analysis and ultimate analysis. The proximate analysis consists of determining the moisture, fat, protein, sugar, starch, ash and crude fibre or dietary fibre. The suitability of different chemical analysis techniques for determining different food constituents is briefly discussed. However, the ultimate analysis that refers to the determination of a particular element or a compound present in the material is out of the scope. The physico-chemical properties of oils and fats are also included in this unit.

12.6 KEY WORDS

D r y i : A n o

ng

peration in which water is removed from a food produc

t under the current of dry air or under vacuum in an

oven.

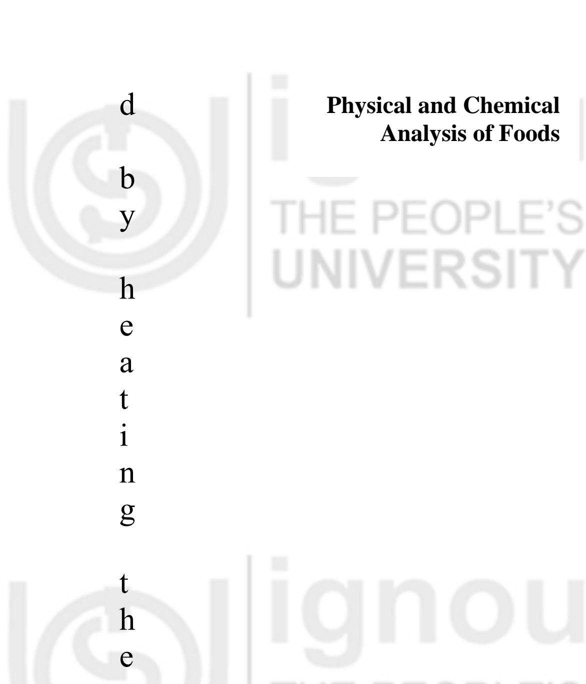
Refluxing : An operation in which a part of the product str

eam that is returned to the chemical process during h

eating to assist in giving increased conversion or re

covery.

Distillation : The process of producing a vapour from a liqui



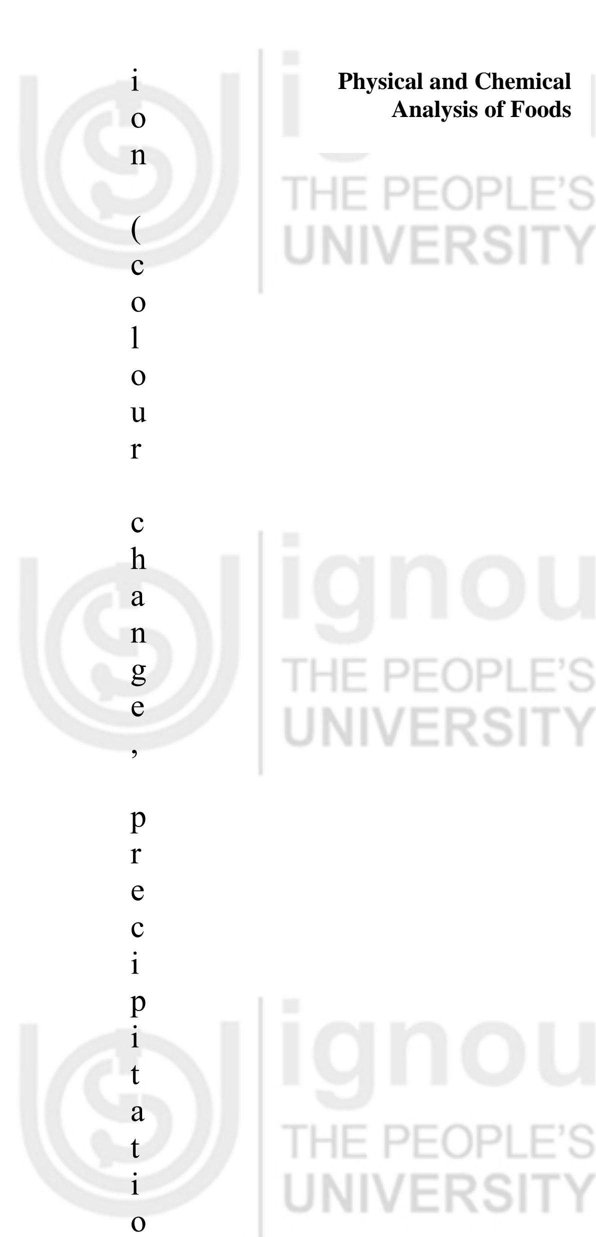
d by heating the liquid in a vessel and collecting an

d condensing the vapours into liquid.

Titration : A method of anal

yzing the composition of a solution by adding known a

mounts of a standardized solution until a given react



ion (colour change, precipitation, or conductivity ch

ange) is produced.

Vapour Pressure : For a liquid or solid, the pressure

of the vapour in equilibrium with the liquid or soli

d at a particular temperature.

Biuret : A condensation product

of urea, also known as carbomyl urea ($NH_2CONHCONH_2$).

Oxidation-reduction Indicator : A compound whose colour in the oxidized state differs

from that in the reduced state.

Volatile Oil : An oil with the tende

ncy to become vapour generally found by the distillat

ion of spices or condiments. Its components are mainl

y terpenes, benzene derivatives. etc.

12.7 TERMINAL QUESTIONS

1) Describe the importance of filth in food products? Briefly discuss the method of determination of filth?

2) Discuss the method of gradation of cereals according to the size of grains?

3) Describe at least three different techniques for the measurement of moisture in food products?

4) Explain the different factors responsible for the error in protein determination by Kjeldahl method?

5) Discuss the different steps involved in the determination of total dietary fibre in foods?

6) Describe the effect of water activity on different physico-chemical properties of foods?

7) How can you determine starch in foods containing higher content of sugar?

8) Name the different physico-chemical parameters oil by which we can evaluate the purity of oil?

12.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) Filth is any objectionable matter contributed by animal contamination of product such as rodent, insect or bird matter or any other objectionable matter contributed by insanitary conditions. Heavy filth can be separated by sedimentation based on different densities of filth, after immersing food particles in solvents like chloroform.

2) Specific heat is the number of calories (heat) required to raise the temperature of one gram of substance by 1°C. Heat = Weight × specific heat × change of temperature.

3) Ostwald viscometer is suitable for measuring the viscosity of liquid food product with low consistency. Ostwald viscometer is a capillary viscometer which gives more accurate result than any other viscometer.

4) The specific gravity of a substance is the ratio of its weight to the weight of an equal volume of another substance taken as standard while density is the ratio of mass to a fixed volume of a material at a particular temperature.

5) The surface tension is defined as the force acting upon a line of unit (1cm) length in the surface of the liquid. The falling drop method is a simple method used to measure the surface tension of a liquid.

Check Your Progress Exercise 2

Your answer should include following points:

1) More frequently moisture in food products is determined by methods like oven drying, vacuum drying, distillation, Karl Fischer titration, etc.

2) The Kjeldahl method for protein determination in foods follows three basic steps: (a) Digestion – decomposition of organic matter by heating in the presence of concentrated sulphuric acid, the end result is ammonium sulphate solution. (b) Distillation – Ammonium sulphate is converted into gaseous ammonia by addition of an excess base, followed by boiling and condensation of the ammonia in a receiving solution. (c) Titration – quantification of the ammonia released from the digest.

3) The water activity is not determined by the total quantity of water in a sample but only by that fraction which is least tightly bound.

4) Dietary fiber is separated from other constituents of food by means of enzymatic hydrolysis while crude fiber is separated by means of acid and alkali hydrolysis of other food constituents.

5) The ash content is an indicator of product quality and the nutritional value of food products; e.g. milk powder with high ash content indicates adulteration with alkali neutralizer.

6) In Lane and Eynon titration method for sugar estimation, methylene blue is used as an oxidation-reduction indicator of the end point. Its use is based on the fact that it is reduced and completely decolourized by minute amounts of reducing sugar or invert sugar but not so long as any cupric salt is present.

Check Your Progress Exercise 3

Your answer should include following points:

1) The important physical parameters used for the quality evaluation of oils/fats are specific gravity, refractive index, melting point, colour, etc.

2) Iodine value is defined as the number of g of iodine absorbed per 100 g of the oil or fat under specified conditions. Iodine value indicates the level of unsaturation in fat.

3) RM value is defined as the number of ml of 0.1N NaOH solution required to neutralize the steam volatile water soluble fatty acids distilled from 5 g of oil or fat under the precise conditions. This is determined by saponifying fat by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulphuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

4) Definitely rancid fat proclaims itself by odour and taste but it is not so simple to determine the presence of rancidity in the early stages of development. The rancidity in fat or oil can be evaluated either by Kreis test or by determining peroxide value.

12.9 SOME USEFUL BOOKS

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UNIT 13 INSTRUMENTATION IN FOOD ANALYSIS

Structure

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- 13.1.2 Why do We Need Instrumentation in Food Analysis?

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- 13.2.1 Criteria for Selecting a Technique
- 13.2.2 Instrumental Techniques in Food Analysis

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13.12 Answers to Check Your Progress Exercises
13.13 Some Useful Books

13.0 OBJECTIVES

After reading this unit, we shall be able to:

- reason advanced instrumentation required in food analysis;
- outline principles of instrumental techniques used in food analysis; and
- describe applications of instrumental technique in analysis of macro and micro food components.

13.1 INTRODUCTION

13.1.1 Need for Food Analysis

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents. All food products whether raw or processed are analyzed to provide information about a wide variety of different characteristics, including their composition, structure, physicochemical properties and sensory attributes. In fact the food is analyzed for a variety of reasons, e.g. compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, detection of adulteration, research and development.

Food safety is an issue of prime importance. With the growing concerns about the food and health safety, the food regulatory authorities in different countries have imposed stringent mandatory norms for the presence of various toxicants, which if present beyond a prescribed residual level might prove hazardous to human health. Moreover, with the implementation of WTO and globalization, it has become important that all food products for export out of the country should meet the regulatory norms of the prescribed limits of different toxicants in various food products. As, government bodies regulate the permitted levels of contaminant compounds; much of this advancement has been driven by increased sensitivity and specificity of determination technique e.g. using analytical instruments.

Food Analysis serves as a unique and invaluable tool for all food scientists, technologists and regulatory authorities for quality assurance and control of food products, to study the different aspects of food products.

Food is a complex matrix consisting of different components. These components can be categorized into different categories which are listed as given below:

1) **Nutrients:** e.g. Proteins, Amino acids, Total cholesterol, Trans Fats and Lipid profile, Carbohydrates, Sugars, Dietary fiber, Vitamins, Minerals etc. Depending upon the food product some of them may be present at high concentration levels while others may be present at low concentration levels of parts per million.

2) **Additives:** e.g. Colors, Dyes, Stabilizers, Antioxidants, Flavors and Fragrance, Preservatives, etc.

   The additives are added to the food products for the purpose of giving the food products desired appearance, texture, flavor and extending the shelf life. The additives are usually present at very low concentration levels.

3) **Adulterants:** They are added intentionally to the food products mostly for the purpose of cost benefits and they may be present at higher as well as lower amounts. They may be safe or sometimes highly toxic, such as, argemone in mustard oil, sudan red in chillies, animal cholesterol in ghee, low cost vegetable oil in high cost vegetable oil etc.

4) **Contaminants and Toxicants**: Toxicants can be classified into Physical toxicants (e.g. glass, wood, metal, insect matter etc.); Biological toxicants such as microbes and pathogens; and Chemical toxicants such as residual pesticides, residual antibiotics, mycotoxins, and environmental pollutants like PAH (polycyclic aromatic hydrocarbons), PCB (polychlorinated

biphenyls), Dioxins, toxic metals etc. Most of the times these contaminants are not added intentionally but find there way into the food products from environmental pollution or if proper practices are not being followed during agriculture, animal breeding, storage or processing. The various toxicants are present at low levels of concentration and if present beyond a certain prescribed level of concentration in food products may prove to be highly toxic or carcinogenic to humans.

13.1.2 Why do We Need Instrumentation in Food Analysis?

Due to complex nature of food matrix, it often becomes impossible to accurately analyze one component in the presence of others using the classical method of analysis. More often than not, interferences are encountered during the measurement of minor components in the presence of the components present in bulk quantities. All this may lead to inaccurate and unreliable results and sometimes erroneous and false results because of lack of specificity and sensitivity of classical method. Therefore, in order to achieve the reliability of results, today the instrumental analytical techniques have become mandatory in development, quality control and safety, exports of food products and meeting the regulatory norms of food products.

13.2 SELECTING AN APPROPRIATE INSTRUMENTAL TECHNIQUE

There are usually a number of different analytical techniques available to determine a particular property of a food material. It is therefore necessary to select the most appropriate technique for the specific application. The analytical technique selected depends on the property to be measured, the type of food to be analyzed and the reason for carrying out the analysis.

13.2.1 Criteria for Selecting a Technique

Some of the criteria that are important in selecting an instrumental analytical technique are listed below:

- **Precision:** A measure of the ability to reproduce a result by a specific analyst (or group of analysts) using the same equipment and experimental approach kepping other conditions unchanged.
- **Reproducibility:** A measure of the ability to reproduce result using the same experimental approach in same as well as different laboratories using same/different equipment.
- **Accuracy:** A measure of how close one can actually measure the value to the true value of the parameter being measured.
- **Simplicity of operation:** A measure of the ease with which relatively unskilled workers may carry out the analysis.
- **Speed:** Analysis of single sample or the number of samples in a given time.
- **Sensitivity:** A measure of the lowest concentration of the component that can be detected by a given procedure.
- **Specificity:** A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components e.g., Fructose in the presence of sucrose or glucose.

- **Nature of food matrix:** The composition, structure and physical properties of the matrix material surrounding the analyte often influences the type of method that can be used to carry out an analysis e.g. whether the matrix is solid or liquid, transparent or opaque, polar or nonpolar.

If there are a number of alternatives methods available for measuring a certain property of a food, the choice of a particular method will depend on which of the above criteria is most important.

13.2.2 Instrumental Techniques in Food Analysis

Analysis of food products for the majority of the parameters can be undertaken using different instrumental techniques as described below.

- **Chromatographic Techniques**
- **Hyphenated Techniques**
- **Spectroscopic Techniques**
- **Thermal methods of analysis**

13.3 CHROMATOGRAPHIC TECHNIQUES

Chromatography is defined as a process by which solutes are separated by a dynamic differential migration in a system consisting of two or more phases, one of which moves continuously in a given direction called as mobile phase and in which the individual substances exhibit different mobilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The individual substances thus separated can be identified or determined using appropriate technique. Chromatographic techniques can broadly be classified into:

1) Gas Chromatography (GC)

Gas chromatography is applied to volatile organic compounds. The mobile phase is a gas and the stationary phase is usually a liquid on a solid support or sometimes a solid adsorbent.

2) Liquid Chromatography (LC)

Liquid chromatography is used to separate analytes in solution including metal ions and organic compounds. The mobile phase is a solvent and the stationary phase is a liquid on a solid support, a solid, or an ion-exchange resin.

13.3.1 Gas Chromatography

Gas Chromatography (GC) is one of the most versatile analytical techniques used in the food industry. GC is used to separate volatile organic components in a mixture. It enables fast separation and identification of components in a complex mixture using appropriate detectors. Substances to be analysed by GC must be volatile and must be thermally stable below 350°C.

A schematic diagram of a gas chromatograph showing various components is presented in Fig. 13.1. Samples are rapidly injected by means of a hypodermic micro syringe through a silicone rubber septum into the column. The sample injection port, column, and detector are heated to temperatures at which the

sample has a vapour pressure of at least 10 torr, usually about 50ºC above the boiling point of the highest boiling solute. The injection port and detector are usually kept at higher temperature than the column to promote rapid vaporization of the injected sample and prevent sample condensation in the detector. For packed columns, liquid samples of 0.1 to 10 µL are injected. For capillary columns, volumes of only about 1/100 these sizes must be injected because of the lower capacity (albeit greater resolution) of the columns. Sample splitters are included on chromatographs designed for use with capillary columns that deliver a small fixed fraction of the sample to the column, with the remaining part going to waste.

The response is detected in the form of peaks. Separation occurs as the vapor constituents equilibrate and partition between carrier gas and the stationary phase. The carrier gas is a chemically inert gas available in pure form such as argon, helium, or nitrogen.

The sample is automatically detected as it emerges from the column (at/or a constant flow/ gas pressure rate), using a variety of detectors whose response is dependent upon the composition of the vapor. The chromatographic peaks are recorded as a function of time. By measuring the **retention time** (elapsed time in minutes between the time a sample is injected and the time the chromatographic peak reaches maximum intensity) and comparing this time with that of a standard of the pure substance, it may be possible to identify the peak (agreement of retention times of two compounds does not guarantee the compounds are identical). The area under the peak is proportional to the concentration, and so the amount of the substance can be quantitatively determined. The peaks are often very sharp and, if so, the peak heights can be compared with a calibration curve prepared in the same manner.

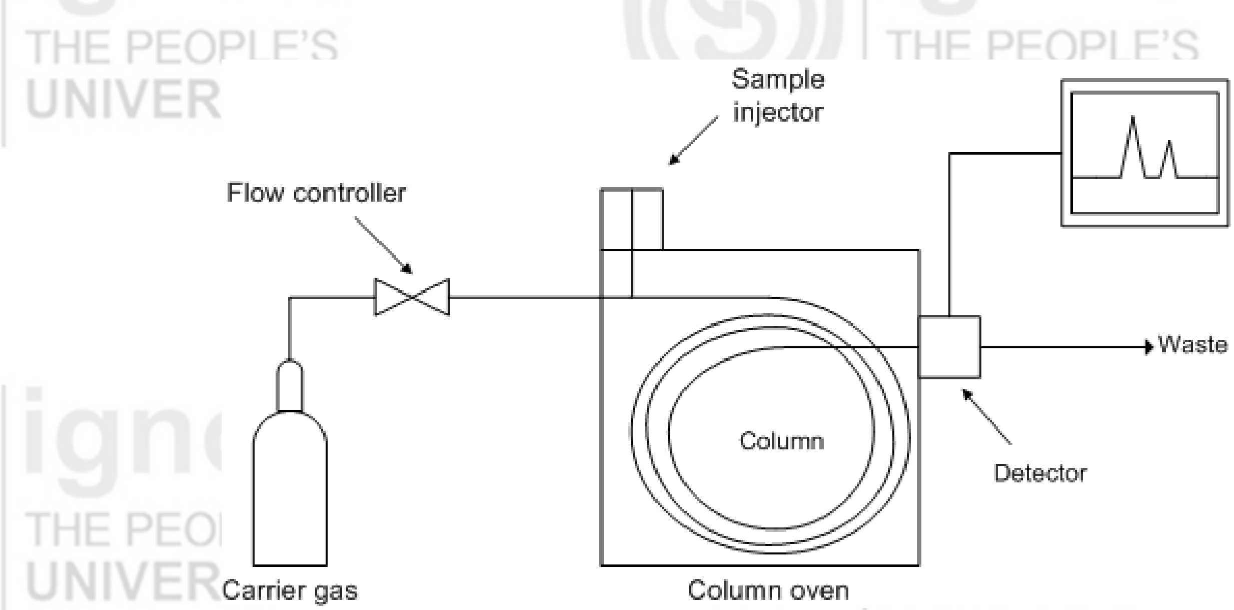

Fig 13.1: Schematic Diagram of a Gas Chromatograph

13.3.2 Detector for Gas Chromatography

Various types of detectors are used for different applications:

- Flame Ionisation Detector (FID)
- Electron Capture Detector (ECD)

- Nitrogen Phosphorous Detector (NPD)
- Thermal Conductivity Detector (TCD)

A) **Flame Ionisation Detector (FID):** FID is the most widely used of all detectors because, it is reliable, easy to maintain and operate, rugged and responds to almost all classes of compounds but, insensitive to water. The response is roughly dependent upon number of carbon atoms in the molecule. Hydrogen and air are the required gases for the ignition of flame.

An organic compound is burnt in a flame produced by H_2 and air which in turn produces ions.

Organic sample + H_2 + O_2 -----> CO_2 + H_2O + $(ions)^+$ + e^-

The ions produced are collected by a pair of polarized electrodes inside the detector and the current is produced which is directly proportional to the concentration. The current is amplified and then recorded.

B) **Electron Capture Detector (ECD):** ECD is the most sensitive detector for halogenated compounds, anhydrides, peroxide, conjugated carbonyls, nitrites, nitrates and organometallics, but, insensitive to hydrocarbons, alcohols, ketones and amines. Solvent containing analyte from the column is passed over a β (electron)-emitter (radioactive compound that emits electrons, e.g. ^{63}Ni). Electron from the emitter causes ionization of carrier gas producing a burst of electrons, which produce a constant current between a pair of electrodes. But, when a solute with higher electron effinity is eluted from the column, some of the electrons are captured and the current flow is reduced in proportion to the concentration of the eluting compound.

C) **Nitrogen Phosphorous Detector (NPD):** NPD is a selective detector for nitrogen and phosphorous containing organic compounds, e.g. organophosphorus and carbamate pesticides. NPD is similar in design to FID. In this detector nitrogen and phosphorus containing molecules exiting the column collide with electrically heated ceramic thermoionic bead which is positioned near the jet orifice and undergo catalytic surface chemical reaction. Ions created in this reaction are attracted towards a collector electrode, amplified and output is given to data system.

D) **Thermal Conductivity Detector (TCD):** This detector is used for the analysis of permanent gases. It responds to all gases and vapours with a thermal conductivity different from that of carrier. In this detector, no separate fuel is required. This detector consists of two pair of heated elements, each as an arm of a Wheatstone bridge over which the column effluent and a reference gas stream flow. When there is only reference gas, balance of conductivity in the bridge is maintained. But when the analyte reaches the filament, the thermal conductivity is changed, causing unbalanced bridge which provide signal.

13.3.3 Sampling Techniques

There are different types of sampling techniques normally employed for GC analysis of volatile samples:

1) Headspace analysis.

2) Thermal Desorption.

3) Purge and Trap technique

1) Headspace Analysis

A sample in a sealed vial is equilibrated at a fixed temperature, for example 10 min. and the vapour in equilibrium above the sample is sampled and injected into the gas chromatograph flavour analysis etc.

2) Thermal Desorption

Thermal Desorption (TD) is a technique in which solid or semisolid samples are heated under a flow of inert gas. Volatile and semi volatile organic compounds are extracted from the sample matrix into the gas stream and introduced into a gas chromatograph. Samples are typically weighed into a replaceable PTFE tube liner, which is inserted into a stainless steel tube for heating.

Thermal desorption is well suited for dry or homogenous samples such as food packaging films, spices, coffee flavour profile, voletile organics in wine, mushrooms, fruits, honey etc. solid foods, cosmetics, ointments, and creams.

3) Purge and Trap Technique

The Purge and Trap technique is a variation of thermal desorption analysis in which volatiles are purged from a liquid sample placed in a vessel by bubbling a gas (e.g. air or nitrogen) through the sample and collecting the volatiles in a sorbent tube containing a suitable sorbent. The trapped volatiles are then analyzed by thermally desorbing them from the sorbent. This is a form of 'Headspace' analysis in which analytes are concentrated prior to introduction into the GC.

Purge and Trap is suitable for non-homogenous samples, since fairly large and high moisture samples can be taken. Examples include foods such as pizza or fruits. The measurements of malodorous organic volatiles in the headspace vapor above a sample of stored food, is used to determine whether it still meets the "freshness" requirements.

13.3.4 Applications of Gas Chromatography

Gas Chromatography can be applied for analysis and determination of different compounds in food products such as :

1) Cholesterol, Fatty acid profiling and Trans fat analysis;
2) Antioxidants and Preservatives like TBHQ, Benzoic acid, Sorbic acid Acetic acid, etc;
3) Analysis of residual pesticides and environmental contaminants;
4) Characterization of flavours and fragrances; and
5) The Gas Chromatographic profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity of the particular oil.

13.4 LIQUID CHROMATOGRAPHY

Liquid chromatography covers a variety of separation techniques such as liquid solid (adsorption chromatography), liquid-liquid (partition chromatography),

ion exchange, size exclusion, thin layer, high performance thin layer and paper chromatography; all involving a liquid mobile phase. In this unit, we would learn more about the liquid chromatographic techniques used specially for the purpose of food analysis i.e.

- High Performance Liquid Chromatography (HPLC)
- Thin Layer Chromatography (TLC)
- High Performance Thin Layer Chromatography (HPTLC)

HPLC is used to separate, identify, and quantify polar and non-volatile compounds when in mixture. HPLC utilizes a column that holds liquid stationary phase, a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.

- **Normal phase chromatography**

In this technique, the stationary phase is polar. A non-polar mobile phase, such as n-Hexane, methylene chloride, or chloroform is used. The stationary phase is bonded silioxane with a polar functional group, e.g. Cyano, diol, amino, dimethylamino, etc. These phases retain polar compounds in preference to non-polar compounds.

- **Reversed phase chromatography**

In Reversed Phase Chromatography (RPC) relatively non-polar stationary phase is used, with a polar mobile phase constituting of methanol, acetonitrile, tetrahydrofuran, etc., usually in combination with water. Methanol and acetonitrile are most common. The water content is varied for adjusting the polarity. Methanol is used for acidic compounds and acetonitrile for basic compounds. Tetrahydrofuran is used for those with larger dipoles. These solvents are UV transparent and have low viscosity. The most common bonded phases are n-octaldecyl (C_{18}) or n-octyl (C_8) bonded polysiloxanes.

13.4.1 Characteristic Features of HPLC

- Fast, accurate and high power of resolution.
- Results are repeatable and reproducible.
- Facilitates determination of multiple components in a single run.
- Provides method of choice for thermally unstable and high molecular weight compounds.
- Separated components can be easily collected from the mobile phase for further characterization, as it is a non-destructive technique.
- Suitable for both aqueous and non-aqueous sample.

13.4.2 Comparison of HPLC and GC

HPLC in some respect is more versatile then GC since:

1) It is not limited to volatile and thermally stable substances. It can accommodate thermally unstable, nonvolatile compounds and inorganic ions.
2) The choice of mobile and stationary phase is wider.

GC is better from the point of view of speed and simplicity of equipment. The analysis cost is also significantly cheaper as HPLC requires highly pure solvents that are costly.

HPLC is an excellent technique for separation of chemical compounds with high degree of specificity and selectivity and is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, as in the case classical column chromatography it is forced through under high pressures of up to 400 atm, which makes it much faster. The technique is not limited by the volatility or stability of the sample compound.

13.4.3 A Typical Modern Liquid Chromatograph

A Typical Modern Liquid Chromatograph Consists of:

i) Solvent delivery system which includes a pump

ii) Sample injection system

iii) Column and Detector

iv) Chromatography software and Computer

Compounds are separated by injecting a sample mixture into the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile and the stationary phase. As the sample solution flows through the column with the mobile phase, the components present in the injected sample solution migrate according to the non-covalent interactions of the compounds with the column. The mobile phase could be run in isocratic and gradient modes. A separation by HPLC, which employs constant composition of mobile phase throughout the chromatographic run, is called an isocratic elution and in case two or more solvents of different polarities are used in different proportions during the chromatographic run the method is known as gradient elution.

The **stationary phase** in HPLC refers to the solid support contained within the column through which the mobile phase continuously flows. Columns containing various types of stationary phases are available commercially. For most of the separation in food analysis the columns normally used are Normal phase (e.g. silica) or the Reverse phase (e.g. C_8, C_{18}). Samples are injected into the HPLC via an injection port. In modern HPLC systems, the sample injection is typically automated.

13.4.4 Detectors for HPLC

In order to detect the compounds as they elute from the column there are many types of detectors that can be used with HPLC. Some of the common detectors include: Ultra Violet (UV), Photo Diode Array (PDA), Refractive Index (RI), Fluorescent, and Mass Spectrometric (MS) detector.

A) **Refractive Index (RI):** Detectors measure the ability of sample molecules to bend or refract light. This property for each molecule or compound is called its refractive index. For most RI detectors, light proceeds through a bi-modular flow-cell to a photo detector. One channel of the flow-cell directs the mobile phase passing through the column while the other directs only the mobile phase. Detection occurs when the light is refracted due to samples eluting from the column, and this is read as a disparity between the two channels.

B) **Ultra-Violet (UV):** Detector measure the ability of a sample to absorb radiation in the UV region. This can be accomplished at one or several wavelengths.

C) **Photo Diode Array (PDA):** Detector produces a three-dimensional graph that assists in examining the purity of a peak. The chromatographic peak is supported by authentication through UV-VIS spectrum.

D) **Fluorescent:** Detector measure the ability of a compound to absorb and then re-emit light at given wavelengths. Each compound has a characteristic fluorescence. The excitation source passes through the flow-cell to a photodetector while a monochromator measures the emission wavelengths.

E) **Mass Spectroscopy (MS) Detectors:** The sample compound or molecule is ionized, passed through a mass analyzer, and the ion current is detected. There are various methods for ionization.

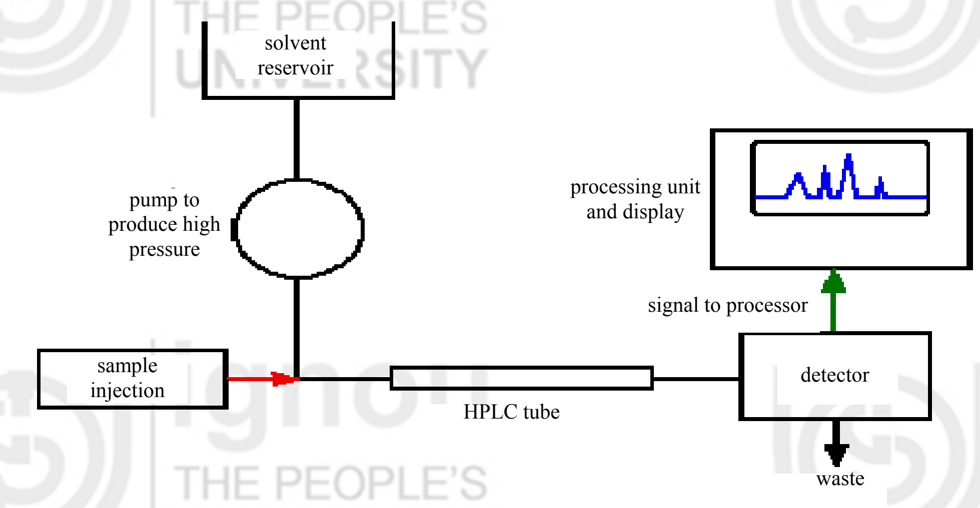

Fig. 13.2: Schematic Diagram of HPLC

13.4.5 Applications of HPLC

HPLC finds wide applications in food, both for profiling and analysis of various components such as:

1) Amino acids profiling, peptides and hproteins.

2) Lipids and alcohols.

3) Carbohydrates and carbohydrate profiling, sweeteners.

4) Fat soluble and water soluble vitamins, carotenoids.

5) Organic acids and organic bases.

6) Residues of Mycotoxin, Antimicrobial and veterinary drugs, pesticides, etc.

7) Pigments, colorants and phenolic compounds.

8) Bittering substances.

9) Additives, preservatives, antioxidants and stabilizers in processed food products.

13.5 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is an important chromatographic technique, frequently used for qualitative identification and initial screening of components. A sample is spotted onto the plate or a strip with a micropipette and the plate or the strip is placed in a suitable solvent to develop the chromatogram. The solvent is drawn up the plate by capillary action, which moves the sample components up the plate at different rates, depending upon their solubility and their degree of retention by the stationary phase. Following development, the individual spots are noted or made visible by treatment with a reagent that forms a coloured derivative. For example, amino acids and amines are detected by spraying the plate with a solution of ninhydrin, resulting in a blue or purple coloured spot. If the solute compound is fluorescent, they can be detected by exposing to UV light. The spots generally move at a certain fraction of the rate at which the solvent moves and they are characterized by the R_f value.

$$R_f = \frac{\text{Distance solute moves from the point of application}}{\text{Distance solvent front moves}}$$

R_f value is characteristic for a given stationary phase and solvent combination. Since the separation and identification spots on TLC is based upon visual observation, at certain times, if the product analyzed contains large number of components, the method may suffer from poor resolution due to the closely lying or the overlapping spots and poor specificity. Therefore, the results may sometimes be uncertain, misleading or inaccurate.

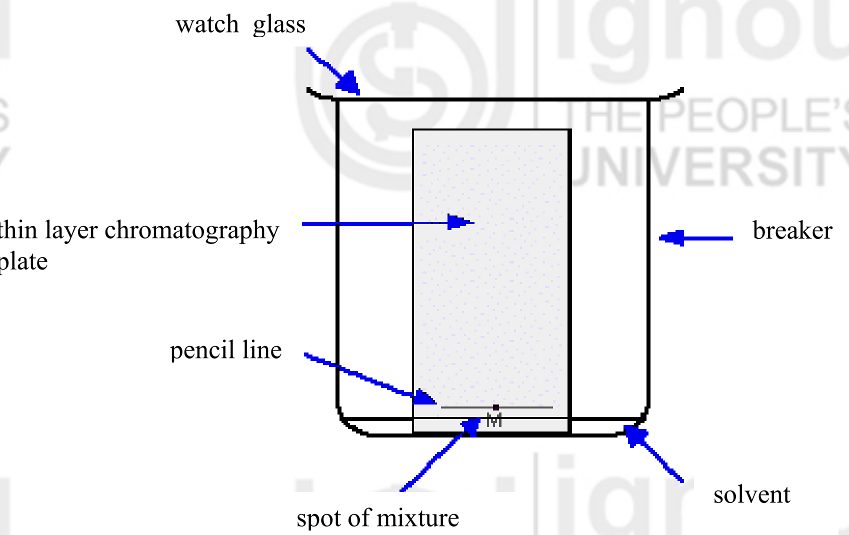

Fig. 13.3: Schematic Diagram of a thin Layer Chromatographic Technique

13.6 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC enhances the power of thin layer chromatography by improving the speed and efficiency of separation by the development of instrumentation to automate sample application, development of the chromatogram, and detection, including accurate and precise *in situ* quantitation.

HPTLC is a selective, precise, and accurate chromatographic technique for fingerprint analysis of food products. This involves densitometric evaluation after resolving the components of the sample on silica gel plates with very fine

particle size. For densitometric evaluation, peak areas are recorded at the appropriate wavelength. HPTLC has the advantages of many fold possibilities of qualitative and quantitative detection in analyzing food products with accuracy and precision.

Applications

HPTLC can be used for a large number of applications in food industry for screening purposes, e.g.:

- Determination adulterants in food products such as argemone in mustard oil.
- Separation of food colours in food samples.
- Determination of residues of mycotoxins in food products.

Check Your Progress Exercise 1

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

1) Define Chromatography technique?

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2) Explain the applications of Gas Chromatography?

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3) Name the detectors used for HPLC and GC.

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13.7 HYPHENATED TECHNIQUES

In the past two decades, combining chromatographic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for the identification and/for confirmation of the identity of target and unknown chemical compounds. For most (trace-level) analytical problems in research field of food products, the combination of column liquid chromatography or

capillary gas chromatography with a mass spectrophotometer has become the preferred approach for the analysis of food products. Two very important examples of hyphenated procedures that are used in food analysis are GC-MS and LC-MS. The information obtained from GC-MS or LC-MS is not only useful for quantitation but also provide confirmation of the analyte, specially if the residue is present at trace level. In GC and HPLC analysis, the results sometimes might be misleading as compounds having similar chemical nature often elute at approximately the same retention time.

Mass spectrometry is an analytical technique that identifies the chemical composition of a sample on the basis of the mass to charge ratio of charged particles. The technique has both qualitative and quantitative uses, including:

- Identifying unknown compounds by the mass and mass fragmentation pattern, which are usually specific in nature. Identification is also possible on comparison to standard mass spectrometry libraries, which usually accompanies GC-MS instruments. In addition, it is also possible to develop own library of standard reference compounds that can be utilized in identifying compounds in unknown samples.
- Determining the isotopic composition of elements in a compound.
- Determining the structure of a compound by observing its fragmentation pattern.
- Quantifying the amount of a compound.

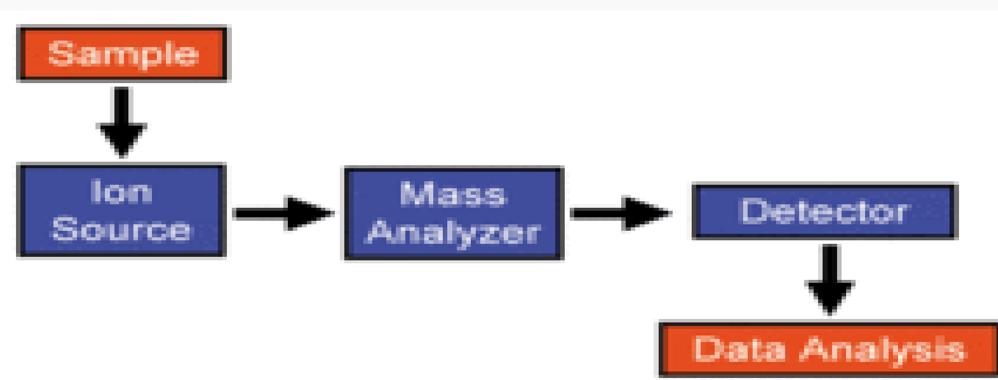

Fig. 13.4: Schematic Diagram of GC-MS

The underlying principle of mass spectrometry is that as charged particles ions pass through electric and magnetic fields, their paths vary according to their mass-to-charge ratios. Devices which operate according to this principle are called mass spectrometers. The design of a mass spectrometer has three essential modules: an **ion source**, which transforms the molecules of a sample into a variety of ionized fragments; an **analyzer**, which sorts the ions by their masses, using internally generated electric and magnetic fields; and a **detector**, which measures the value of some indicator quantity and thus providing data for calculating the abundances of respective ion fragments.

13.7.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The capabilities of integrated gas chromatography-mass spectrometry are almost unique in meeting the requirements for analytical methods which are

not only highly sensitive but also specific and reliable in providing information on specific compounds as a function of their concentrations.

The GC portion of this system provides separation of volatile organic solutes in a mixture in the gas phase. As each solute exits the GC column, it is diverted into a mass spectrometer which is capable of monitoring both the amount and identifying the chemical nature of the solute. In this way, both quantitative and qualitative information about the mixture can be obtained.

The MS portion of the system takes each gaseous solute eluting out of gas chromatograph and ionizes it in an electron beam. The ions formed by a specific solute will depend on the nature of the bonds in the molecule, and it is possible to get both ionized molecules and its fragments. The ions thus formed are then directed down a separator which isolates and counts the ions according to mass. The sequence and relative intensity of the mass peaks give information about the chemical identity of the solute. The absolute intensity of the peaks provide information about the amount of substance present.

Applications of Gas Chromatography Mass spectrometry:

Gas chromatography is useful for a number of applications in food analysis specially from the point of view of safety of the food products. The technique is useful for:

1) Pesticide residue analysis in all raw and processed food products.
2) Analysis of environmental contaminants, such as polyclorinated biphenyls, polyaromatic hydrocarbons, dioxins, etc. in food products.
3) Fatty acid profiling in oils and fats.
4) Flavours and fragrance in food products.
5) Volatiles and other residual solvents in food packaging material.

13.7.2 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry (LC-MS) is an analytical technique that combines the physical separation capabilities of HPLC coupled with the confirmation by mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally, its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals in a complex mixture.

LC-MS technique is used when very high sensitivity and specificity at trace residue level is required.

Types of Mass analyzer used :

There are a different types of mass analyzers that could be used in LC/MS.

- Single Quadrupole,
- Triple Quadrupole,
- Ion Trap,
- TOF (Time of Flight), and
- Quadrupole-time of flight (Q-TOF).

The quadrupole and ion trap instruments are highly sensitive and hence used for target-oriented residue analysis; whereas the TOF and Q-TOF instruments are mainly used for accurate mass analysis and structure identifications of target and non-target compounds, metabolite identifications, etc.

Applications of Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS is routinely used for detection of :

1) Mycotoxins: LC-MS is routinels used for detection of: Toxins produced by different fungi, e.g. *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., etc. Some of the mycotoxins regularly analyzed in food samples include Aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A, etc.
2) Residual drugs and antibiotics in different food products.
3) Banned dyes and colourants e.g. Sudan dyes in different food products.
4) Residual pesticides in raw and processed food products.

13.8 SPECTROSCOPIC TECHNIQUES

Spectroscopy pertains to the dispersion of an object's light into its component colors (i.e. energies) via prism or the study of the interactions between radiation and matter as a function of wavelength (λ). This technique utilizes interactions between electromagnetic radiation and matter to provide information about food properties, *e.g.,* molecular composition, structure, dynamics and interactions. A variety of the instruments that are commonly used to analyze food materials based on spectroscopy includes, UV-visible, fluorescence, atomic, infrared and nuclear magnetic resonance spectrometer.

13.8.1 Distribution of Energy in Atoms and Molecules

Atoms and molecules can only exist in a limited number of discrete energy levels: they cannot have energies between these levels, *i.e.,* their energy levels are **quantized**. Each molecular species has a unique set of energy levels that depends on its unique atomic structure (electrons, protons, neutrons) and molecular structure (type and arrangement of atoms and bonds). The lowest of these energy levels is referred to as the ground state, while higher levels are referred to as *excited states.* The *potential energy* of an atom or molecule is usually defined relative to the ground state (which is arbitrarily taken to have zero energy). The potential energy of a molecule is made-up of contributions from a number of different sources: electronic, vibrational, rotational, translation and nuclear.

- **Electronic Energy Levels:** Electrons in an atom are arranged into a number of different shells and sub-shells. An electron can move from one of these sub-shell levels to another by absorbing or emitting radiation of appropriate energy. The system is then said to have undergone an *electronic transition.* Electronic transitions may involve electrons that are in inner shells (higher energy) or outer shells (lower energy) of atoms.

- **Vibrational Energy Levels:** Molecules (but not atoms) can vibrate in a number of different modes, *e.g.,* the atoms can compress or stretch along the axis of a bond, or they can bend symmetrically or asymmetrically. Each of these vibrations occurs at a characteristic frequency (energy) that depends on the mass of the atoms and the strength of the bonds involved.

- **Rotational Energy Levels:** Molecules often contain chemical groups that are capable of rotating around certain bonds at fixed frequencies (and therefore energies). Each group has a specific number of frequencies at which it rotates and therefore has a specific number of quantized rotational energy levels. The rotation frequency is determined by the mass of the atoms involved and their distance from the axis of rotation.

- **Nuclear Energy Levels:** The nuclei of certain atoms have a property known as *spin.* A (charged) spinning nucleus generates a small magnetic field and can be thought of as a small magnet. Normally, this magnet can be orientated in any direction, but in the presence of an external magnetic field it can only align itself either with or against the field, *i.e.,* it is quantized. Transitions between the different energy levels within the nuclei can be made to occur by applying radiation of a specific energy to the sample.

- **Translational Energy Levels:** Atoms and molecules are in continual translational motion because of the thermal energy of the system. Translational energy levels are quantized, however, the differences between the energy levels are so small that the molecules act as though the energy is distributed continuously.

13.8.2 Characteristics of Electromagnetic Waves

Electromagnetic waves may be thought of as *small packets* of energy (*photons*) that move through space with *wave*-like properties, *i.e.,* they exhibit *wave-particle* duality (e.g. photoelectric effect). They consist of oscillating electric and magnetic fields that are perpendicular to one another, and to the direction of propagation. The sinusoidal variation in the amplitude of the electric vector of the wave can be plotted as a function of time (at a fixed position within a material) or as a function of distance (at a fixed point in time). A monochromatic (single wavelength) electromagnetic wave that propagates through a vacuum can be described completely by its frequency, wavelength and amplitude (or parameters derived from these):

- The *frequency (v)* of a wave is the number of cycles per second (Hz = s^{-1}).
- The *period (T)* of a wave is the time taken to complete a cycle: $T = 1/v$.
- The *wavelength* λ is the distance between successive maxima/minima of a wave.
- The *wave number* (cm^{-1}) is the number of cycles per unit distance.
- The *amplitude* (*A*) of a wave is the maximum magnitude of the electric vector.
- The *intensity* (*I*) of a wave is proportional to the square of the amplitude. It is the amount of energy passing through a given area per second. Increasing the intensity of an electromagnetic wave increases the *number* of quanta passing a given area per second, not the energy of each individual quantum.
- The *velocity* (*c*) of an electromagnetic wave is the distance travelled per second: $c = v\lambda$. The velocity of an electromagnetic wave travelling through a vacuum is the speed of light $c = 3 \times 10^8$ ms^{-1}. The velocity of an electromagnetic wave traveling through a material is always less than that in a vacuum. The refractive index of a material is equal to $c_{vacuum}/c_{material}$.

- The energy (E) of the photons in an electromagnetic wave is related to the frequency of the wave:

  $E = h\nu = h/T = hc/\lambda$

  where, h = Planks constant (6.6262×10^{-34} J s). These expressions can be used to relate the energy of an electromagnetic wave to its frequency, period, wavelength or wave number. This relationship indicates that monochromatic radiation (*i.e.,* radiation of a single frequency) contains photons that all have the same energy.

  The *electromagnetic spectrum* consists of radiation that ranges in wavelength from 10^{-12} m (high energy) to 10^4 m (low energy). The physical principles and mathematical description of radiation across the whole of the electromagnetic spectrum is the same, however, it is convenient to divide it into a number of different regions depending on the origin of the waves, *i.e.,* cosmic rays, gamma rays, x-rays, ultraviolet, visible, infrared, microwaves, and radio waves.

13.8.3 Interaction of Radiation with Matter

Spectroscopic techniques utilize the fact that atoms and molecules have a discrete set of energy levels and that transitions can only occur between them. When an electromagnetic wave propagates through a material, the atoms or molecules can absorb energy and move to an excited state if the photons in the wave have energies that are exactly equal to the difference between two energy levels ($\Delta E = h\nu$). Alternatively, if an excited atom or molecule emits energy in the form of radiation the waves emitted must have energies that are exactly equal to the difference between two energy levels ($\Delta E = h\nu$). The energy of the photons in different regions of the electromagnetic spectrum corresponds to different types of energetic transitions that can occur in atoms and molecules, *e.g.,* electronic, rotational, vibrational, translational, nuclear transitions. Electromagnetic radiation can therefore be used to probe different molecular characteristics of matter. The atomic or molecular origin of the transitions that occur between different energy levels in matter, the region of the electromagnetic spectrum that these transitions correspond to, and the spectroscopic techniques that can be used to measure these transitions are summarized below:

Transition	**Region of e/m Spectrum**	**Spectroscopy Technique**
Electronic (̃ıkJ mol^{-1})	UV-Visible	UV, Visible, Atomic Fluorescence
Vibrational (10 kJ mol^{-1})	Near and Mid Infrared	Infrared
Rotational (0.1 kJ mol^{-1})	Far Infrared, Microwaves	Infrared
Nuclear (10^{-6} kJ mol^{-1})	Radio Waves	Nuclear Magnetic Resonance (NMR)

The difference between *electronic* energy levels is greater than between *vibrational* energy levels, which is greater than between *rotational* energy levels. Thus higher energy radiation (shorter wavelength) is needed to cause transitions between electronic levels than between vibrational or rotational levels. In practice, a molecule can be thought of as having a number of different electronic energy levels, with rotational and vibrational energy levels superimposed on them.

I) Absorption

Absorption is the process by which energy is transferred from an electromagnetic wave to an atom or molecule and causes it to move to an excited state. Absorption can only occur when an atom or molecule absorbs a photon of light that has energy, which exactly corresponds to the difference between two energy levels, *i.e.,* it must be quantized. At room temperature, the ground state of atoms and molecules is usually the most probable and stable state and hence transitions usually occur from the ground state to higher energy levels. At higher temperatures, more of the higher energy levels are occupied and so, transitions between higher energy levels may also become important.

If an atom or molecule is subjected to electromagnetic radiation of different wavelengths (energies) it will only absorb photons at those wavelengths which correspond to exact differences between two different energy levels within the material. A plot of the fraction of photons absorbed at a particular wavelength versus the energy of the photons at that wavelength is called an *absorption spectrum.* Conventionally, the axis of absorption spectra are specified in terms of easily measurable quantities: *x*-axis shows transmittance or absorbance (rather than fraction of photons absorbed); *y*-axis shows wavelength, frequency or wave number (rather than photon energy).

II) Emission

Emission of radiation is the reverse of absorption, occurring when energy from an atom or molecule is released in the form of a photon of radiation. When a molecule is raised to an excited state it will only exist in this state for a very short time before relaxing back to the ground state. This is because it will always try to move to its lowest energy state. There are two important relaxation processes through which an excited molecule can dissipate its energy:

- **Non-radiative decay:** This is the most common way that an excited molecule loses its energy. Energy is dissipated in a number of small (quantized) steps due to transfer of energy from the excited molecule to surrounding molecules in the form of kinetic energy (heat). Nevertheless, the heat generated is usually so small that it has little effect on the overall temperature of the system.

- **Radiative decay:** In some cases, an atom or molecule loses its energy in the form of a photon (emission). This is the case in atomic emission spectroscopy.

Sometimes both of these processes occur together. In fluorescence spectroscopy, a molecule absorbs electromagnetic radiation, which causes it to move into an excited state. It then returns to the ground state by dissipating some of its energy in the form of non-radiative decay and the rest in the form of a photon of radiation. The photon emitted is therefore of lower energy

(longer wavelength) than the incident wave. Usually, an electron decays to the lowest energy level in the excited electronic state, and then returns to the ground state.

13.8.4 Measurement Modes

The design of an analytical instrument based on spectroscopy depends on the nature of the energetic transitions involved (*e.g.,* electronic, vibration, rotation, translation, nuclear), the nature of the radiative process involved (*e.g.,* absorption, emission, fluorescence) and the nature of the food matrix (*e.g.,* absorbing or non-absorbing). These factors determine the wavelength (frequency) of electromagnetic radiation used, the way that the electromagnetic radiation is generated and the way that the electromagnetic radiation is detected. Some commonly used designs are highlighted below:

- ***Emission***: The sample being analyzed is energetically stimulated (*e.g.,* by heating or application of radiation) and the amount of electromagnetic radiation produced by the sample is measured at different wavelengths, *e.g.,* atomic emission spectroscopy, NMR, fluorescence.
- ***Transmission***: An electromagnetic wave generated by the analytical instrument is propagated directly through the sample and the reduction in its amplitude due to interaction with the sample is measured at different wavelengths, *e.g.,* atomic absorption spectroscopy, infrared transmission measurements, UV-visible spectrophotometery.
- ***Reflection****:* An electromagnetic wave generated by the analytical instrument is reflected from the surface of the sample and the reduction in its amplitude due to interaction with the sample is measured at different wavelengths, *e.g.,* infrared reflection measurements, color measurements.

13.9 SPECTROSCOPIC INSTRUMENTS

The most commonly used spectroscopic instruments for food analysis are:

- UV- Visible Spectroscopy
- Atomic-Absorption Spectroscopy (AAS)
- Inductively Coupled Plasma (ICP)
- Nuclear Magnetic Resonance Spectroscopy (NMR)
- Fourier Transform Infrared Spectroscopy (FTIR)

13.9.1 UV-Visible Spectroscopy

UV-visible spectroscopy is an important tool for the chemical profiling of food products after extraction of the components in the suitable solvent. Chemical profiling using W-visible spectroscopy complemented with chemical profiling with other chromatographic technique can be used as reference for quality control of food products.

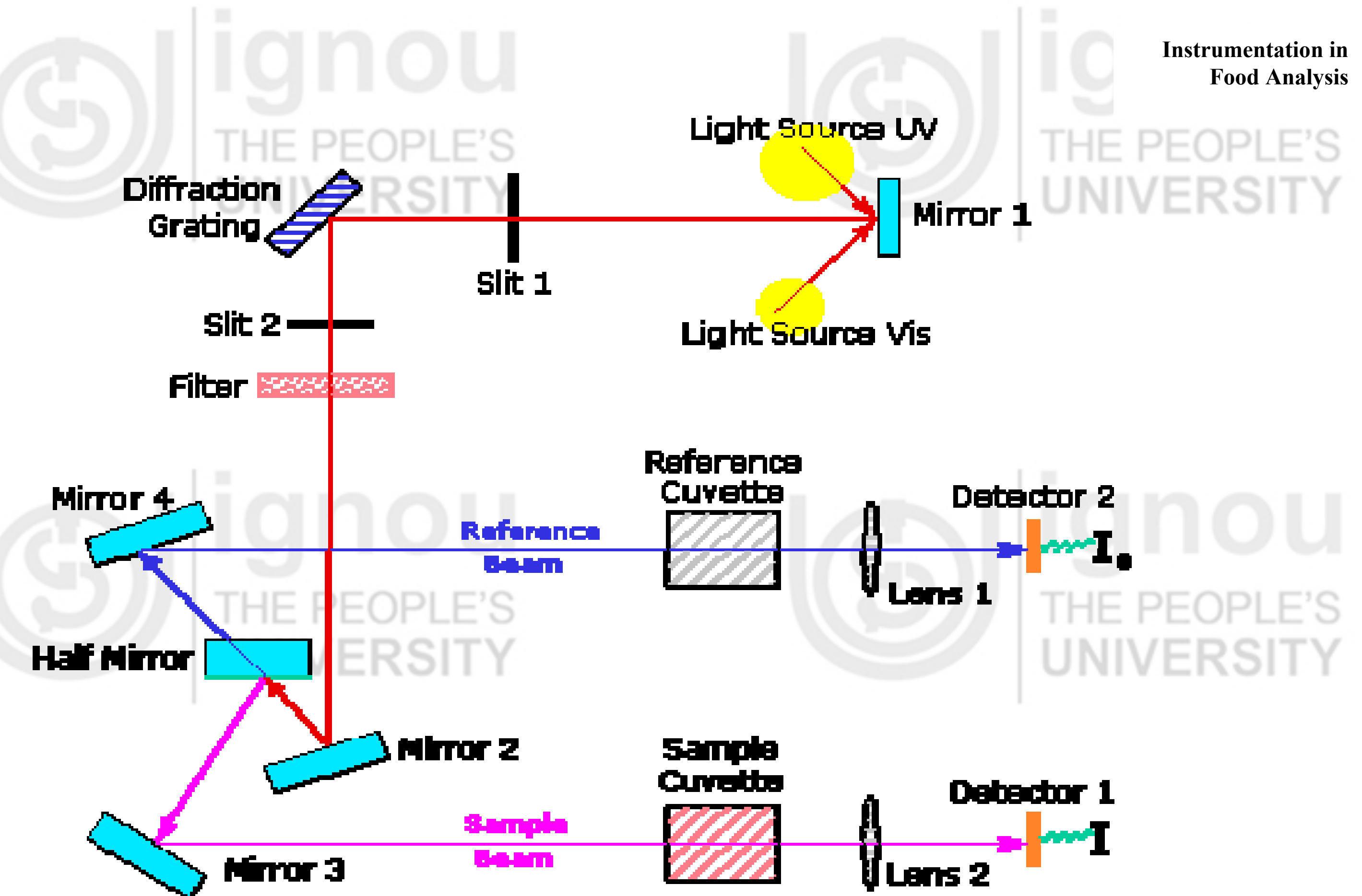

Fig. 13.5: Schematic Diagram of UV-visible Spectroscopy

A beam of light from a visible and/or UV light source is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as $\mathbf{I_0}$. The intensity of the sample beam is defined as **I**. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm. If the sample compound does not absorb light of a given wavelength, $\mathbf{I} = \mathbf{I_0}$. However, if the sample compound absorbs light then **I** is less than $\mathbf{I_0}$, Absorption may be presented as **transmittance** ($\mathbf{T} = \mathbf{I/I_0}$) or **absorbance** ($A$= log $\mathbf{I_0/I}$). If no absorption has occurred, T = 1.0 and A= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as λ_{max}. Different compounds may have very different absorption maxima and absorbances.

The most commonly used solvents are water, ethanol, hexane and cyclohexane. Solvents having double or triple bonds, or heavy atoms are generally avoided.

13.9.2 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectrometry is a very popular method for assessing the concentration of metals and minerals that may be present in the food products. This technique allows measuring all the elements of periodic table. It encompasses a wide variety of techniques and provides rapid, sensitive and selective determination of elemental composition.

Atomic absorption spectrometer has five basic components, which are:

1) A light source (cathode lamp)
2) A sample cell (absorption cell)
3) Monochromator
4) Detector
5) Output unit

In this technique, the elements in the sample are brought into their ionized form in solution by using the wet digestion, dry ashing or suitable microwave assisted digestion system and then aspirated through a nebulizer into the high temperature flame where the sample gets converted into gaseous atoms. The source of light is usually a hollow cathode lamp, which is composed of the element being measured. Each element requires a different lamp. The hollow cathode lamp produces emission lines specific for the element used to construct the cathode. The lamp is filled with an inert gas like argon or neon. When a potential is applied, the gas is ionized and is driven towards the cathode and cause the metal atoms to sputter all the surface of the cathode and produce specific atomic emission lines.

The characteristic emission lines produced by the source i.e. hollow cathode lamp are absorbed by the atoms which get excited and are raised to higher energy level. As the sample passes through the flame, the beam of light passes through the monochromator. The monochromator isolates the specific spectrum line emitted by the light source through spectral dispersion and focuses it upon a photo multiplier detector where light signal is converted into an electrical signal. The process of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for readout or further fed into a data station.

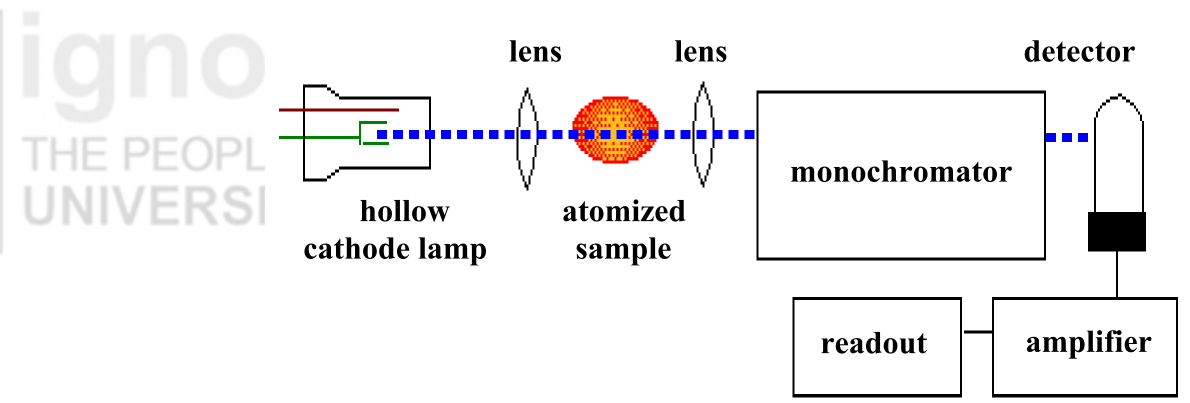

Fig. 13.6: Schematic of an Atomic-Absorption Experiment

The greater amount of sample present, the higher the absorbance energy. Different flames can be achieved by using different mixtures of gases,

depending on the desired temperature and burning velocity. Some elements can only be converted to atoms at high temperatures. Even at high temperatures, if excess oxygen is present, some metals form oxides that do not re-dissociate into atoms. To inhibit their formation, conditions of the flame may be modified to achieve a reducing, non-oxidizing flame. The most widely used flames are air-acetylene and nitrous oxide-acetylene flame. The nitrous oxide-acetylene flame has a higher temperature as compared to air-acetylene flame.

A) Electrothermal Atomization

In determination of certain elements (Ag, Be, Ca, Fe, Pb and Sn), flame atomic absorption spectroscopy may not be very sensitive. Use of electro thermal atomization provides sensitivity up to the levels of parts per billion (ppb) to subparts per billion levels. This type of atomization requires a graphite furnace, which is a graphite tub, where the sample is rapidly atomized after thermal pre-treatment. To maintain a dense fraction of free ground state elements in the optical path, an inert gas atmosphere is used. Since the dilution and expansion effects of flame cells are avoided, and the atoms have a longer residence time in the optical path, a higher peak concentration of atoms is obtained.

B) Vapour Generation- Atomic Absorption Spectroscopy (AAS-VGA)

Vapour Generation- Atomic Absorption Spectroscopy (AAS-VGA) is used for the determination of hydride-forming elements, such as As, Hg, Sn, etc., using quartz tube atomizer. VGA-AAS is highly sensitive and effective method for quantification of toxic metals in food products and can be used for the determination of these elements at ppm to ppb levels.

Applications of Atomic-absorption spectroscopy

1) Atomic absorption spectroscopy can be used to analyze the concentration of esssential minerals and toxic metals in all raw and processed food products.

13.9.3 Inductively Coupled Plasma – Optical Emmission Spectrophotometry (ICP-OES/MS)

Inductively Coupled Plasma (ICP) is an analytical technique used for the detection of trace metals in Food samples. The primary goal of ICP is to get elements to emit radiations of characteristic wavelength that can then be measured.

An ICP requires that the elements, which are to be analyzed, in the solution form. An aqueous solution is preferred over an organic solution, as organic solutions require a special accessory prior to introduction of a sample into the ICP. Solid samples are also discouraged, as clogging of the instrument can occur. The nebulizer transforms the aqueous solution into an aerosol. The light emitted by the atoms of an element in the ICP must be converted to an electrical signal that can be measured quantitatively

An ICP typically includes the following components:

- Sample introduction system (nebulizer)
- ICP torch
- High frequency generator

- Transfer optics and spectrometer
- Computer interface

ICP is an atomic emission technique using argon plasma as an excitation source. The sample is introduced into a premix spray chamber, where it is directed up the central tube of the ICP ''torch''. The torch consists of concentric tubes with independent argon streams flowing through each tube. The top of the torch is centered within a Radio Frequency (RF) induction coil, which is the source of energy for the system. After ignition, the plasma is propagated through inductive coupling with the RF field generated from the coil. The ICP torch is designed specifically to promote penetration of the plasma skin by the sample, allowing sample atoms to experience the full energy of the plasma source. The high temperatures provided by the ICP provide excellent sensitivities for refractory elements and also essentially eliminate chemical interferences. Like all emission techniques, there are no source lamps. By monitoring several wavelengths, either all at once or in a programmed sequence, many elements can be determined in one automated analysis. ICP emission, therefore, offers significant speed advantages over atomic absorption for multi element analysis. Except for the refractory elements, which may be substantially better than even graphite furnace AA, ICP detection limits are comparable to flame atomic absorption. The high temperatures of the ICP carry one disadvantage. The plasma is so effective in generating excited state species that the rich emission spectra produced increase the probability of spectral interferences. High resolution monochromators and sophisticated software for background and inter element correction are used to deal with this potential problem. Another limitation of ICP emission is the initial cost of the instrument. The price for basic ICP systems starts at about the same level as the prices for top-of-the-line automated AA systems. More sophisticated instrument can cost two to four times the price of basic systems.

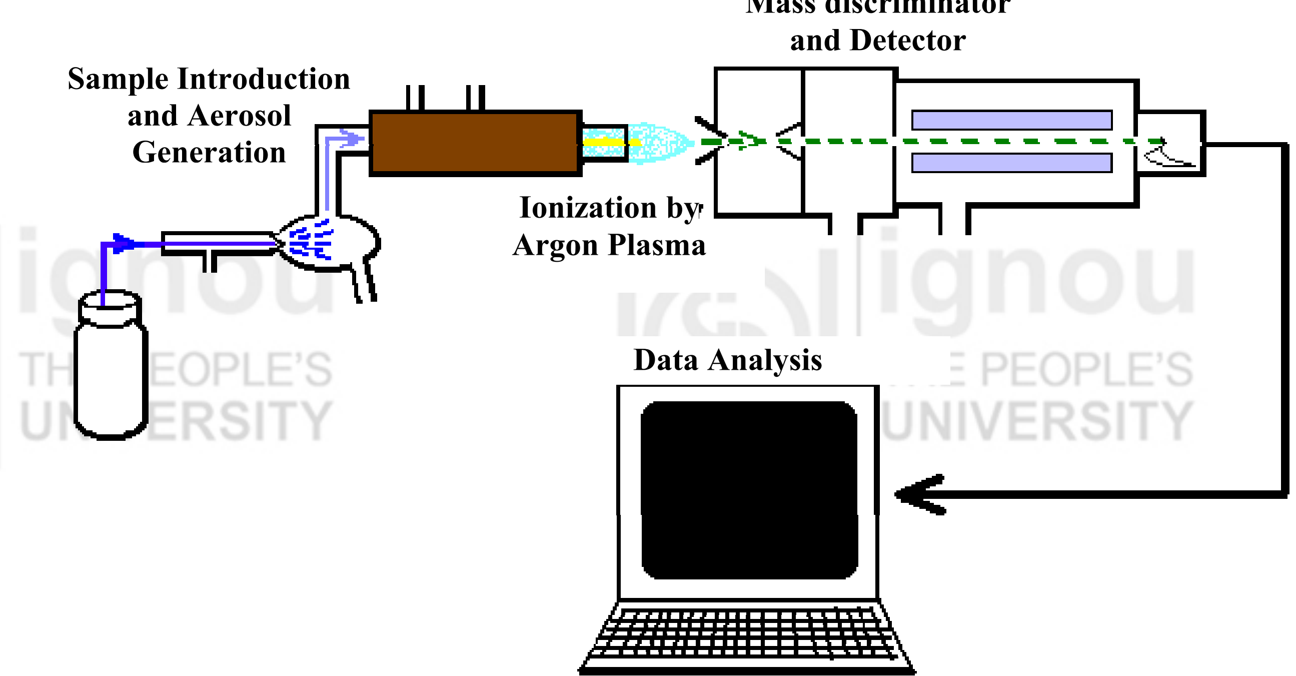

Fig. 13.7: Schematic Diagram of ICP – OES

13.9.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectroscopy exploits the magnetic properties of certain nuclei. The most important applications for the organic chemist are proton NMR and carbon-13 NMR spectroscopy. In principle, NMR is applicable to any nucleus possessing spin.

Much information can be obtained from an NMR spectrum. Analysis of a NMR spectrum provides information on the number and type of chemical entities in a molecule. A bit more detail + enclosures followed.

The impact of NMR spectroscopy on the natural sciences has been substantial. It can, among other things, be used to study mixtures of analytes, to understand dynamic effects such as change in temperature and reaction mechanisms, and is an invaluable tool in understanding protein and nucleic acid structure and function. It can be applied to a wide variety of samples, both in the liquid and the solid state.

13.9.5 Fourier Transform-Infrared Spectroscopy (FT-IR)

The mid-IR region covers the range from 4000 to 400 cm^{-1}. The basic principle of the IR technique is that various organic functional groups absorb infrared light at specific wavelengths. Thus, since every organic molecule has a unique chemical composition, it also has a unique infrared spectrum. Biological samples are composed of proteins, carbohydrates, lipids and nucleic acids. Since these molecules contain functional organic groups, the IR spectrum consists of bands from all these components.

The infrared spectrum is very complex, and it contains a large amount of information. To evaluate the data, it is necessary to use multivariate statistical analysis.

Some of the applications of FT-IR Spectroscopy for food analysis include:

1) Characterization of essential oils in various spices
2) Evaluation of trans fats in oils and fats

Check Your Progress Exercise 2

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

1) What do you understand by Hyphenated techniques? List any two examples of this technique?

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2) Which Spectroscopic technique is used for analyzing the concentration of metals and minerals in food products?

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3) What are the application of FT-IR Spectroscopy for food analysis?

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13.10 THERMAL METHODS OF ANALYSIS

Most foods are subjected to variations in their temperature during production, transport, storage, preparation and consumption, e.g. pasteurization, sterilization, evaporation, cooking, freezing, chilling etc. Temperature changes cause alterations in the physical and chemical properties of food components which influence the overall properties of final product e.g., taste, appearance, texture and stability. Chemical reaction such as hydrolysis, oxidation or reduction may be enhanced, or physical changes, such as evaporation, melting, crystallization, aggregation or gelation may occur. A better understanding of the influence of the temperature on the properties of food enables food manufacturers to optimize processing condition and improve product quality. It is therefore important for food scientist to have analytical technique to monitor the changes that occur in food. These techniques are often grouped under the general heading thermal analysis, at present the term thermal analysis is usually reserved for a narrow range of technique that measures changes in the physical properties of foods with temperature e.g. mass, density, rheology, and heat capacity.

A variety of differential analytical technique have been deployed to monitor changes in the physical properties of food components that occurs in response to controlled changes in temperature. A number of most important of these thermal analysis techniques are described below:

13.10.1 Thermogravimetry

Thermogravimetry technique continuously measure the mass of a sample as it is heated or cooled at a controlled rate, or it is held at a particular temperature for a period of time with reference to a control sample. Thermogravimetry is useful for monitoring process that involves a change in the mass of food or food component, e.g., drying, liberation of gasses, absorption of moisture. To study the various types of processing and storage condition that a food might normally experience, thermogravimetric instruments have been specially designed to allow measurements to be carried out under specific environments e.g., controlled pressures or atmosphere. Gravimetric instruments typically consist of a sensitive balance situated within a container whose pressure, temperature and gaseous environment can be carefully controlled. The ability to carefully control the temperature, pressure and composition of the gases surrounding a sample is extremely valuable for food scientist, because it allows them to model processes such as drying, cooking, and uptake of moisture during storage.

Differential Thermal Analysis and Differential Scanning Calorimetry

DTA and DSC are techniques rely on changes in the heat absorbed or released by a material as its temperature is varied at a controlled rate. These changes occur when components within a food undergo some type of phase transition

(e.g., crystallization, melting, evaporation, glass transitions, conformational change) or a chemical reaction (e.g. oxidation, hydrolysis)

13.10.2 Differential Thermal Analysis (DTA)

DTA is defined as a technique for recording the difference in temperature between a substance and a reference material against time or temperature as the two specimen are subjected to identical temperature regimes in a environment heated or cooled at a controlled rate. A typical instrument consists of two measurement cells that are located in a temperature controlled environment, whose temperature can be varied in a programmed manner. The sample to be tested is placed into the 'sample cell' while a reference material of known thermal properties (often distilled water) is placed in the "reference cell". The two cells are then heated or cooled at a controlled rate. The small difference in temperature between a "sample cell" and "reference cell" ($\Delta T = T_{sample} - T_{reference}$) is measured using accurate thermocouples placed below the cells as the temperature of the external environment ($T_{external}$) is varied in a controlled fashion. The output of the instrument is therefore a plot of ΔT versus $T_{external}$. The position of the peak provides information about the temperature that the transition occurs. The under a peak depends on the amount of material involved in the transition and the enthalpy change per unit amount of material.

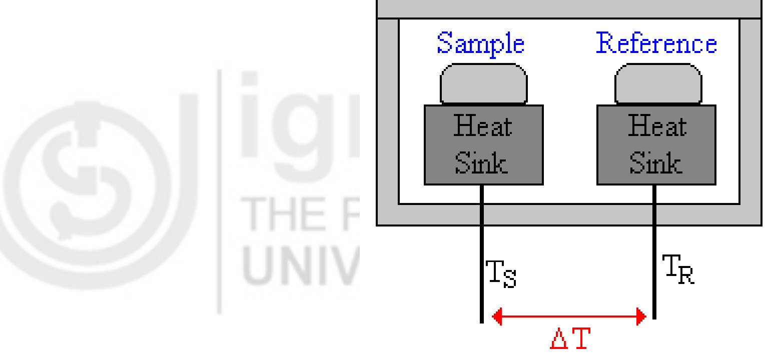

Fig. 13.8: Differential Thermal Analysis

13.10.3 Differential Scanning Calorimetry (DSC)

DSC is a technique for recording the energy required to keep a zero temperature difference between a sample cell and a reference cell that are heated or cooled at a controlled rate. The thermocouples constantly measure the temperature of each cell and heaters supply heat to one or other of the cells so that they both have exactly the same temperature. If a sample were to undergo a phase transition it would either absorb or release heat. DSC data is therefore reported as the rate of energy absorption (Q) by the sample relative to the reference material as a function of external temperature. Information about thermal transitions that occur within a sample is obtained by analyzing the Q versus T external thermo gram. It should be noted that it is possible to measure the change in the heat release by a material as a function of time under isothermal (constant temperature) conditions.

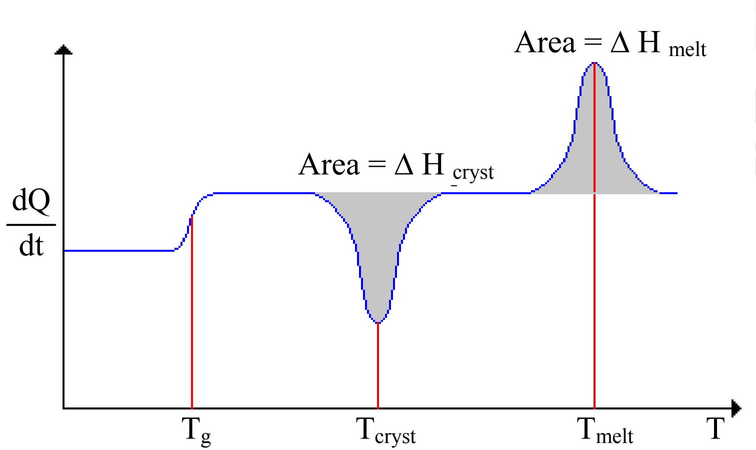

Fig. 13.9: An illustrated Thermogram

Applications

- **Determining Specific heat capacity:** The Specific heat capacity is an important physical quantity in food industry, since it is the amount of energy that must be supplied or withdrawn from a material in order to increase or decrease its temperature by a 1ºC. The knowledge of Specific heat capacity of a material is therefore important in the design of processes such as chilling, freezing, warming, sterilization and cooking.

- **Phase transitions:** DSC and DTA are routinely used in the food industry to characterize phase transitions in foods, for e.g. crystallization, melting, glass transitions and conformational changes. They can be used to provide information about the temperature at which transitions occur. When a material changes its physical state from solid to liquid (melting) or from liquid to solid (crystallization) it absorbs or gives out heat respectively. Pure substances usually have very sharp melting or crystallization points and therefore all the heat is absorbed or evolved over a narrow range of temperatures, leading to sharp DSC or DTA peak. Many food components are chemically complex materials and therefore the phase transitions occur over a wide range of temperature, e.g. edible oils contain a wide variety of different triacylglycerols each with its own melting points. Peaks from food oils may also be complicated by the fact that triacylglycerols can crystallize in more than one different crystalline structure i.e. they are polymorphic.

Check Your Progress Exercise 3

Note: a) Use the space below for your answers.

b) Check your answers with those given at the end of the unit.

1) Name different types of Thermal analysis methods used in food?

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2) Describe briefly the various applications of Thermal method of analysis in food?

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13.11 KEY WORDS

Chromatography	:	It is a technique where solutes are separated by a dynamic differential migration in a system consisting of two or more phases where one is mobile phase and the other is a stationary phase.
Spectroscopy	:	Spectroscopy pertains to the dispersion of a light into its component colors (i.e. energies) via prism or the study of the interaction between radiation and matter as a function of wavelength (λ).
Mass Spectrometry	:	Mass spectrometry is an analytical technique that identifies the identity of a chemical substance on the basis of the mass to charge ratio of fragmented charged particles.
HPLC	:	It is a separation technique with a solid stationary phase (column) and a liquid mobile phase.
GC	:	It is a separation technique with a solid stationary phase (column) and gaseous mobile phase. Generally used for volatile compounds analysis.
AAS	:	Atomic spectroscopy is the study of absorption and emission characterictics of geseous atoms of elements.
ICP	:	Elements are excited by Argon plasma to gaseous ions which are further identified and quantified by emission spectroscopy or mass spectrometry.
Rheological Technique	:	It is the study of deformation and flow in food or material in response to an applied load or deformation.
Hyphenated Technique	:	It is the combination of two or more analytical techniques, e.g. chromatographic separation system (HPLC/GC) on-line with a spectroscopic detector (MS), in order to obtain structural information of the analytes present in a sample.

NMR Spectroscopy : NMR spectroscopy is the technique of studying structural properties of compounds such as type and nature of bonds using the magnetic properties of certain nuclei possessing spin.

Wheatstone Bridge : It is an electrical circuit. In wheat-stone bridge four resistance R_1, R_2, R_3 and R_4 are connected end to end with each other to form a closed loop. A sensitive glavanometer "G" is connected between their junctions. The circuit is provided with two keys "K_1" and "K_2". Generally wheat-stone bridge is used to determine unknown resistances.

13.12 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) It is a technique where solutes are separated by a dynamic differential migration in a system, consisting of two or more phases where one is mobile phase and the other is a stationary phase.

2) Gas Chromatography can be applied for analysis and determination of different compounds in food products such as:

   a) Cholesterol, Fatty acid profiling and Trans fat analysis;

   b) Antioxidants and Preservatives like TBHQ, Benzoic acid, Sorbic acid Acetic acid, etc;

   c) Analysis of residual pesticides and environmental contaminants;

   d) Characterization of flavours and fragrances; and

   e) The Gas Chromatographic profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity of the particular oil.

3) **Detector used for gas chromatography are:**

   Flame Ionisation Detector (FID), Electron Capture Detector (ECD),

   Nitrogen Phosphorous Detector (NPD) Thermal Conductivity Detector (TCD)

   Detector used for HPLC are:

   Ultra Violet (UV), Photo Diode Array (PDA), Refractive Index (RI), Fluorescent, and Mass Spectrometric (MS) detector.

Check Your Progress Exercise 2

Your answer should include following points:

1) It is the combination of two or more analytical techniques, e.g. chromatographic separation system (HPLC/GC) on-line with a spectroscopic detector (MS), in order to obtain structural information of the analytes present in a sample.

   Examples: GC-MS, LC-MS

2) Atomic Absorption Spectroscopy

3) Applications of FT-IR Spectroscopy for food analysis include:

   a) Characterization of essential oils in various spices.

   b) Evaluation of trans fats in oils and fats.

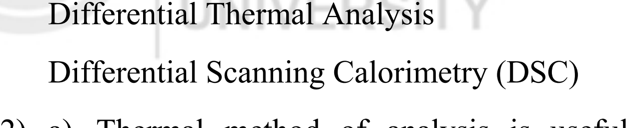
Check Your Progress Exercise 3

Your answer should include following points:

1) Thermogravimetry

   Differential Thermal Analysis

   Differential Scanning Calorimetry (DSC)



2) a) Thermal method of analysis is useful in measuring specific heat capacity of a material.

   b) DSC and DTA are used to characterise phase transition in foods e.g. crystallization, melting, glass transition and conformational changes.

13.13 SOME USEFUL BOOKS

S.S. Nielsen, (1998). *Introduction to Food Analysis*. Aspen Publishers - The best general overview of food analysis techniques currently available. .

Y. Pomeranz and C.E. Meloan. *Food Analysis: Theory and Practice*. Chapman and Hall - General overview of food analysis techniques.

D.W. Gruenwedel and J.R. Whitaker. *Food Analysis: Principles and Techniques*. Marcel Dekker - General overview of food analysis techniques.

C.S. James. *Analytical Chemistry of Foods*. Blackie Academic and Professional - General overview of food analysis techniques.

Official Methods of Analysis, Association of Official Analytical Chemists - Officially recognized methods of analysis for many food components.

UNIT 14 SENSORY EVALUATION OF FOOD PRODUCTS

Structure

14.0 Objectives
14.1 Introduction
14.2 Selection of Panel
  14.2.1 Types of Panel
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14.0 OBJECTIVES

After reading this unit, you would be able to:

- underline the importance and significance of sensory evaluation;
- enlist quality control aspects of sensory evaluation;
- Evaluate and assess the quality of food products based on the sensory parameters; and
- know the relevance and significance of sensory evaluation of food products and use sensory evaluation as a tool for improvements in quality of food products besides creating the consumer acceptance.

14.1 INTRODUCTION

As we all know, the sensory behavior of food products is the ultimate criterion for the acceptability of any product by the consumer. Unless the food products meet the desired standards of taste, flavor, texture, etc., the consumer will not accept the products. In other words, quality of food products to a consumer means the sensory behavior of products. Overall quality of food products depends on factors such as quantity, nutritional parameters, physico-chemical and physico-mechanical parameters, several other hidden attributes and sensory properties. At the time of buying any food product, we should look for nutritional parameters, like calories, the vitamins, the minerals, the proteins, and other ingredients, etc. At the same time, we also remain concerned with the presence of undesirable substances in the food products, for example, all the toxic and allergic ingredients. Since the undesirable constituents in food products may cause serious health hazards, usually, these parameters are regulated by stringent government guidelines and norms. Labelling of nutritional parameters is required by law in most countries.

The most important parameter for both the processors and consumers is the sensory quality. As the name suggests, the term 'sensory' is related to senses of the human being. Sensory quality is important to processor, since it attracts consumers but it is equally important to the consumers, since it satisfies their aesthetic and gustatory senses.

Keeping in view, the importance of the sensory quality, the sensory attributes needs to be evaluated. Evaluation of sensory quality has been defined as "*a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, taste, touch and hearing*".

Sensory evaluation of products is assuming increasing significance, as it provides information for several purposes i.e. quality control, assessment of process variation, cost reduction, product improvement, shelf-life, new product development and analysis of market.

To derive maximum benefits out of sensory evaluation, it is necessary to follow the methodology in its full scientific perspective. The basic steps to perform the sensory analysis are:

a) Selection of the proper panel;

b) Maintaining suitable environmental conditions and use of standard equipment for the test;

c) Obtaining representative samples;

d) Preparation and presentation of samples for evaluation in a manner that ensures the uniformity and representation of the samples; and

e) Selection of the proper methods and statistical techniques.

14.2 SELECTION OF PANEL

Sensory evaluation is normally carried out by designed experiments under proper environmental conditions by both trained and untrained panels. The experts who have acquired the product-specific skills are not appropriate for the general evaluation because of the risk of being biased.

Panels with different degree of training are required for different types of sensory analysis. The degree of training required depends on a number of considerations, such as degree of differences to be detected, number of panelists required for the tests and time and value of the analysis to the product type. Training is also necessary for descriptive and profile panels using special procedures for specific products and situations.

For any sensory evaluation test, at least ten members should be selected in a panel. The panel members should be recruited based on various criteria such as:

a) Interest and motivation

Candidates who are interested in sensory analysis and who have the liking for the test product or products to be investigated are likely to be more motivated

and hence, such kind of people are likely to become better assessors than those who are without such essential interest and motivation.

b) Attitude

Candidates who are quite flexible in their eating habits should be considered. Those with rigid attitudes with strong dislikes and likes towards food products may not be suitable to become a member of the panel.

c) Knowledge and aptitude

Candidates having capacity to concentrate and those who can remain unaffected from all the external influences and those having adequate knowledge of all aspects of the product should be considered as panelists.

d) Health

The candidates must have good general health and should not have any disabilities, which may affect their senses.

e) Ability to communicate/Personality characteristics

The candidates must have ability to communicate and describe the sensations they have perceived while assessing a product. The candidates should be reliable and honest in their approach.

14.2.1 Types of Panel

A) Trained Panel (Laboratory Panel)

The candidates should be carefully selected and trained and it need not be an expert panel. Trained panels provide answers to two general questions relating to the sensory properties of foods:

- Is there a difference between or among stimuli?, and
- What are the direction and the intensity of differences?

The trained panels should ideally have 5 to 10 members and the same should be used in all developmental and processing studies. The panel should be able to establish the intensity of a sensory characters of overall quality of a food. The panel for flavor profile studies should have a higher degree of training for detailed analysis of the flavor spectrum of complex processed foods.

B) Discriminative and Communicative (also known as D and C) Panel (Semi-Trained Panel)

This type of panel should be constituted of people familiar with quality of different classes of foods and thus is capable of discriminating differences and communicating their reactions. The panelists may not be trained formally but they should be capable of following instructions given at the evaluation session. The panel should consist of about 25 to 30 members and should be used to find the acceptability of preference of final products as a preliminary screening programme to select a few for large scale consumer trials.

C) Untrained Panel (Consumer Panel)

The members of the untrained panel should be selected at random from the potential consumers in a market area. The number of panelists should be large enough to ensure due representation of different age, sex, race and income level group in the population of potential consumers. The findings should be based on at least 100 independent judgements.

D. Qualifications for Panelists

The panelists particularly for the trained and Discriminative and Communicative (D&C) panels should have the following qualifications:

a) Sound health without any defects affecting sensory perception;

b) Average sensitivity;

c) Capability of independent judgement;

d) Ability to concentrate, train and learn;

e) Intellectual curiosity and interest in quality evaluation work;

f) Willingness to spend time in evaluation and submission to periodic tests;

g) Freedom from prejudices in respect of a particular food product; and

h) Food enthusiast having the liking for trying different types of foods.

14.2.2 Methodology for Sensory Evaluation

Prior to the start of the sensory evaluation process, the following steps needs to be accomplished:

a) Screening

The panel members should be selected keeping in view the products to be evaluated. The prospective members should appear for a test designed so as to pick the members of desired level of sensitivity.

b) Training

The panel members should undergo a period of training in the type of work they shall be doing later. The members should be educated in special vocabulary and they should be taught to be able to appropriately perceive and express the sensory reactions. Testing sessions should be preceded by a few informal orientation sessions in which the type of sample is introduced and discussed and tentative decisions made about testing conditions, temperature, quantity, mode of presentation, etc. Further, the language used to describe the character notes of aroma, taste and overall quality should be developed and tested. Reference standards for expressing amplitudes should also be discussed in these orientation sessions.

c) Briefing of Panel

The panel members should be given clear and precise instructions before they start testing. When a quality attribute is evaluated, the instructions should be given in the scorecard. In case of rating tests, the panelist should be given clear and precise instructions in respect of scale used to help anchor judgements in respect of degree and direction of quality attributes and grade specification. The instructions should not lead the panel to the identity of particular samples or induce errors of anticipation.

Check Your Progress Exercise 1

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

1) Describe Sensory Evaluation?

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2) What is a sensory panel?

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3) List the types of sensory panel?

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14.3 MAINTAINING SUITABLE ENVIRONMENTAL CONDITIONS

14.3.1 Laboratory Set-up and Equipment

Fig.14.1 shows a typical layout of the sensory laboratory.

a) General

Environmental factors, where the sensory evaluation is to be done and samples which need to be evaluated, should be suitably controlled. Sensory evaluation should be conducted in quiet and well-lit rooms, free from any odors. The rooms should be constructed to have comfort for concentrated prolonged testing and ease of cleaning. Pleasing neutral shades and maintenance of comfortable temperature and humidity conditions of the whole area or at least the room where panel members are going to sit and discuss are desirable and appealing. All these are essential to help the panel members develop interest for carrying out the test. The testing area where booths are located should be separated from sample preparation and wash rooms.

b) Reception and Briefing Room

As the name suggests, the reception and briefing room is the place where the panel members are entertained first. This room should be well maintained and equipped with comfortable chairs. It should be designed to ensure maintenance of pleasant attitudes and it should minimize the congestion to the booths. Panel members should assemble here and to start with, the panel members should be briefed about the test, etc. here.

c) Panel Booths

These areas are the actual places for the tests. The booths should be located between or adjacent to the reception and preparation rooms and should consist of test booths of identical design, a separate table having natural daylight or illuminated with special daylight bulbs for evaluation of colors of samples and a table. The entry and exit to the panel booth area through independent doors is often useful to avoid any communication between panel members, which may lead to any bias while assessing the sample.

d) Preparation Room

The preparation room should be suitably separated from the testing room and it should be equipped for preparing and serving samples. The room should have facilities for cooking of samples with additional facilities for prepared food storage cabinets -hot and cold. The kitchen ventilation should be such that cooking odors are expelled from the laboratory and should not penetrate the panel-booth area.

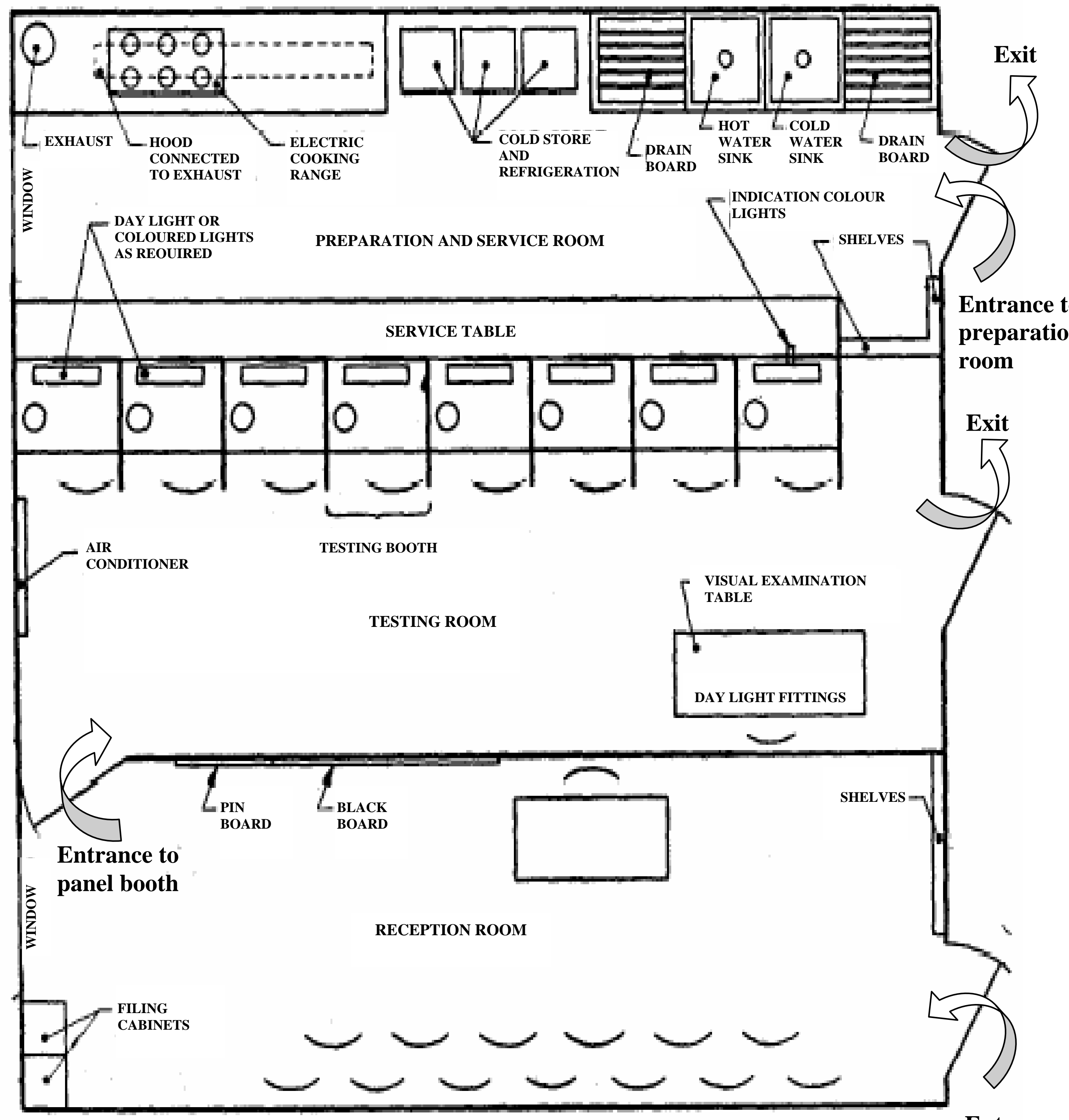


Fig. 14.1: Layout of a typical sensory laboratory

14.4 SAMPLE PREPARATION

Samples should be prepared in a way to bring out the difference in a particular quality attribute under evaluation. All variables like temperature, time of boiling, quantity and composition of water, blending, etc., should be controlled to ensure identical method of preparation for all samples. Care should be taken that no loss of flavor occurs and no foreign tastes or odors are imparted by the procedure during preparation, storage, serving, etc.

14.5 TYPES OF TESTS

The sensory tests may be broadly classified into two major categories i.e.:

1) **Analytical tests:** Analytical tests are based on the evaluation of differences in clarity, quality and/or quantity of sensory characteristics of a product. The panelists for carrying out the analytical tests are screened for interest, ability to discriminate differences and reproduce results. They are trained to function as a human analytical instrument

2) **Affective tests:** Affective tests are based on the evaluation preferences and/or acceptance and/or opinions of product.

   Both these categories have been summarized in Table 14.1

14.5.1 Analytical Tests

Analytical tests are used for laboratory evaluation of products in terms of differences or similarities and for identification of sensory characteristics. There are two major types of analytical tests – discriminative and descriptive. Both the tests employ experienced and/or trained panelists. Potential panelists are screened for selected personal traits, interests and ability to discriminate differences and generate reproducible results.

Table 14.1: Types of Tests for Sensory Evaluation

Classification of methods by function	Appropriate methods	Type and No. of panelists
A. ANALYTICAL TESTS		
(a) Discriminative *(i)* ***Difference test*:** Measures simply the difference between the methods.	• Paired-comparison • Duo-trio • Triangle • Ranking • Rating difference/scalar difference from control	• Normal sensory acuity. • Panel size depends on product variability and judgement reproducibility. • A recommended minimum number is generally 5, since any fewer could represent too much dependence upon one individual's responses.
(ii) ***Sensitivity test:*** Measures the ability of individuals to detect sensory characteristics	• Threshold • Dilution • Rating difference/ scalar difference from Control	
(b) Descriptive Measures qualitative and/ or quantitative characteristics	**Attribute rating** • Category scaling • Ratio scaling (Magnitude Estimation) • Flavor profile analysis • Texture profile analysis • Quantitative descriptive Analysis	• Sensory acuity • Motivated • Trained or highly trained

B. AFFECTIVE TESTS		
	• Paired-performance • Ranking • Rating - Hedonic (verbal or facial) scale - Food action scale	• Randomly selected • Untrained • Representative of target population • Consumers of test product • No recommended "magic number" – minimum is generally 24 panelists, which is sometimes considered rough product screening; 50-100 panelists usually considered adequate

a) Discriminative Tests

There are two types of discriminative tests; difference and sensitivity test. Difference tests measure whether samples can be differentiated at some predetermined level of statistical probability. Sensitivity tests measure the ability of individuals to detect sensory characteristics.

(i) Difference test : There are several types of Difference Tests.

- ***Paired Comparison Test***

Two coded samples are evaluated simultaneously or sequentially in a balanced order of presentation. This test is used to find simple difference and directional difference in a specific characteristic and difference preference in consumer analysis of foods. This is also applicable in training and testing of panelists.

In simple difference test, panelists are asked to test whether the samples in each pair are the same or different. Whereas, in case of directional difference, the panelists are asked to indicate which sample in the pair has greater or lesser degree of intensity of a specified sensory attribute.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the paired comparison tests is given below:

Format 1A: Specimen Evaluation Card For Paired Comparison Test (Simple Difference)

Name: **Date:**

Product: **Time:**

You are given one or several pairs of samples.

Evaluate the two samples in the pair for difference in* ________________.

Indicate your judgement by crossing out words not applicable.

Pair No.	Code No. of Pairs	Your Judgement
01.	-------- ---------	Different/Not different
02.	-------- ---------	Different/Not different

Signature

**The panel organizer should indicate qualify attributes to be evaluated.*

Format 1B: Specimen Evaluation Card For-Paired Comparison
(Directional difference/preference)

Name: **Date:**

Product: **Time:**

You are given one or several pair of samples.

Evaluate the two samples in the pair for difference/preference in* __________.

Indicate your judgement by crossing out words not applicable.

If different, indicate the Code No. of the sample which is more*___ /preferred.

Pair No.	Code No. of Pairs	Your Judgement	If samples in a pair are different, code no. of sample, this is more ___ preferred.
1.	______ ______	Different/Not different	_________________
2.	______ ______	Different/Not different	_________________

Signature

**The panel organizer should indicate the quality attributes to be evaluated.*

The data is analyzed using binomial and multinomial distribution (probability) tables for panel selection, product difference or preference and when and when number of observations exceeds the table value, χ^2-test or t-test for percentage for product difference or preference has to be used.

- ***Duo-Trio Test***

This test employs three samples, two identical and one different. One sample is identified as the standard and presented first, followed by two coded samples, one of which is identical to the standard. The judge is required to identify the sample, which matches the standard.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the duo-trio test is as below:

Format 2: Specimen Evaluation Card For-Duo-Trio Test

Name: **Date:**

Product: **Time:**

⇒ The first sample '*R*' is the reference sample. Test it carefully.

From the pair of coded samples next given, judge which sample is the same as 'R'.

Pair No.	Code No. of Pairs	Code No. of sample Matching with 'R'
1.	__________________	__________________
2.	__________________	__________________
3.	__________________	__________________

Signature

The data is analyzed using binomial and multinomial distribution (probability) tables for panel selection, product difference or preference. When number of observations exceeds the table value, χ^2-test or t-test for percentage for product difference or preference has to be used.

- ***Triangle Test***

This test employs three coded samples, two identical and one different, presented simultaneously. None of the sample is identified as the standard. The judge must determine which of the three samples presented differs from the other two.

The data is analyzed using binomial and multinomial distribution (probability) tables for panel selection, product difference or preference and when number of observations exceeds the table value, χ^2-test or t-test for percentage for product difference or preference has to be used.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the triangle test is as below:

Format 3: Specimen Evaluation Card For-Triangle Test

Name: **Date:**

Product: **Time:**

⇒ **Two of the three samples are identical.**

Determine the odd sample.

Pair No.	Code No. of Samples			Code No. of Odd Sample
1.	______	______	______	____________________
2.	______	______	______	____________________
3.	______	______	______	____________________

Signature

- ***Ranking Test***

This test is used to make simultaneous comparisons of several samples on the basis of a single characteristic. A control needs to be identified; all test samples to be coded. Samples (which may include control or standard) are presented simultaneously and ranked accordingly.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the ranking test is as given below:

Format 4: Specimen Evaluation Card for-Ranking Test

Name: ____________________ **Date:**______________________

Product:__________________ **Time:** _____________________

Please rank the samples in numerical order according to intensity of quality attribute under test of the product or your preference.

Intensity/Ranking Preference	**Code No. or Sample**
First	____________________
Second	____________________
Third	____________________
Fourth	____________________

Comments: (Type of off-flavour, etc.)

Signature

The data obtained from ranking test is evaluated by adopting the following statistical recommendations:

a) If the number of samples exceeds 7, adopt χ^2-test;

b) Rank sum analysis has to be adopted for product difference/preference when the number of observations is within 20;

c) χ^2-test has to be used for product difference/preference;

d) Analysis of variance (ANOVA) is adopted for the ranks converted to normal scores for multiple comparison.

(ii) Sensitivity Test: There are several ways of carrying out sensitivity test.

- ***Threshold test***

These tests are usually expressed as absolute, and indicate the minimum detectable level of concentration of a substance. Criteria of response in determining threshold include *detection threshold* (awareness of change from some neutral background) and *recognition threshold* (point at which the stimulus becomes identifiable).

The identification threshold concentrations (sensitivity of individual panelists) and just noticeable difference values are found from the panel data. The data from the homogeneous panel is used for product evaluation by finding arithmetic or geometric mean according to concentration series given.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the threshold test is as given below:

Format 5: Specimen Evaluation Card for-Threshold Test

Name: ____________________ **Date:**_______________________

Product:___________________ **Time:** ______________________

You receive a series of samples with increasing concentrations of one of the 4 taste qualities (sweet, salty, sour, bitter)*.
Start with Samples No. 1 and continue with Samples No. 2, No. 3, etc.
Retasting of already tested solutions is not allowed.
Describes the taste* and the feeling factors and give intensity scores.

Use the following intensity scale:

0 = None or pure water taste
? = Different from water, but taste quality not identifiable
X = Threshold very weak (identify the taste)
1 = Weak
2 = Medium
3 = Strong
4 = Very strong
5 = Extremely strong

Sample No.	**Description of Taste and Feeling Factors**
1	_____________________
2	_____________________
3	_____________________
4	_____________________
5	_____________________
6	_____________________
7	_____________________
8	_____________________
9	_____________________
10	_____________________
11	_____________________
12	_____________________

Signature

**To be modified for odour analysis.*

- ***Dilution test***

The dilution technique determines the smallest amount of test material that can be detected when it is mixed with a standard material. The technique may

provide information on relative intensities of treatment at comparable dilution levels. Dilution testing is limited to food products that can be made homogeneous without affecting the factor being tested.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the dilution test is as given below:

Format 6: Specimen Evaluation Card for-Dilution Test

Name: ___________________ **Date:**_____________________

Product*:________________ **Time:** _____________________

Assign scores for each sample for various characteristics.

Quality Attributes	**Maximum Score**	**Code No. or Samples**			
Colour	20	_______	_______	_______	_______
Consistency	20	_______	_______	_______	_______
Flavour	40	_______	_______	_______	_______
Absence of defects	20	_______	_______	_______	_______
Total score	100	_______	_______	_______	_______

Comments

Signature

**The weighted rating is a typical score applicable to orange marmelades. For other products similar scales have to be worked out..*

Data from the dilution test is analyzed by finding the arithmetic or geometric mean for the group and expressing as dilution number or dilution index, which is defined as the percentage or ratio of the test substance in one mixture when the substance is just identifiable.

b) Descriptive Tests

Descriptive tests attempt to identify sensory characteristics and quantify them. Panelists are selected on their ability to perceive differences between test procedures.

Descriptive tests are based on two types of methodologies:

i) Attribute rating: It involves:

- **Category Scaling**

Coded samples are presented simultaneously or sequentially in a balanced order, which differs among the individual panel members. Category scales

consists of a series of word phrases structured in ascending or descending order of intensity and are used to measure the specific attributes (e.g. sweetness, off-flavor etc.). For the purpose of analysis, successive digits are later assigned to each point represented on the scale, usually beginning at the end representing zero intensity. A statistical analysis (e.g. analysis of variance) of the mean intensity scores for each sample is used to determine significant differences among the mean scores for the sample represented.

- **Ratio Scaling (Magnitude Estimation)**

This test is used to estimate the relationship between physical intensity and sensory magnitude. It can also be used for comparable ratings on specific attributes among two or more products. The method permits the participants to use a wide range of numbers of his/her own choice with the property that ratios or proportions among the numerical assignments reflect ratios of sensory intensities. The numerical ratings given to the first sample presented may be any one of the subject's choice, except zero or a negative value. Ratings given to the succeeding samples should be in proportion to the rating assigned to the first. The numbers assigned are subjected to statistical analysis after normalization.

- **Flavor Profile Analysis**

The technique provides a written record of a product's perceptible aroma and flavor components, feeling factors and aftertastes. The panelist characterizes individual aroma and flavor notes in the order perceived and assigns an intensity value using a constant rating scale. A panel of four or five members is normally used. Panelists independently examine the product under study, record their impressions of aroma, flavor and aftertastes, then reports to a panel leader in an open discussion. The final flavor profile, upon comparison with an original profile, can show the effect of an ingredient substitution, a processing change packaging, age etc.

- **Texture Profile Analysis**

This is a descriptive technique based on the principle of the flavor profile method. It provides a systematic approach to measure the textural dimensions of food in terms of mechanical, geometrical, fat and moisture characteristics. The panel is composed of six to nine members. The findings of the panels are recorded and a profile for similarities and differences is used for interpretation.

- **Quantitative Descriptive Analysis**

This technique utilizes an unstructured category scale and a panel of not less than six trained panelists, and obtains repeated judgements from each panelist for each test products.

Let us take an example of sensory evaluation for a sample of cookies. In order to evaluate sensory attributes of cookies, following Descriptive Tests can be undertaken:

Texture

Surface 1 — 5 — 10
Smooth — Rough

Dryness 1 — 5 — 10
Oily — Dry

Moisture absorption: 1 — 5 — 10
None — Much

→ **Scale**

Flavor

Sweet 1 — 5 — 10

Salty 1 — 5 — 10

Bitter 1 — 5 — 10

→ **Scale**

14.5.2 Affective (Preference and Acceptance) Tests

Affective tests refer to preference testing based on the measurement of preference, or a measure from which relative preference may be determined, e.g. pleasure-displeasure, like-dislike.

Preference may be defined as:

a) Expression of higher degree of liking, and

b) Choice of one object over others.

Preference is only one of the many factors involved in acceptance.

Acceptance may be defined as:

a) An experience or feature of experience, characterized by a positive attitude; and/or

b) Actual utilization of the product.

Acceptability is inferred from scale ratings.

There are three types of affective tests; Paired – Performance Test, Ranking Test and Rating Scale.

i) Paired – Performance Test

In the simplest application of a paired-performance test for preference, two samples are presented, simultaneously or sequentially. The Panelist is requested to express a preference based on the specific attributes, the reason for preference may be included if desired. The method may also be applied to make multiple paired – comparisons within a sample series, i.e. a standard product vs each of several experimental products.

ii) Ranking Test

This is an extension of the paired-preference test approach. Three or more coded samples are presented simultaneously, sufficient in amount so that the panelist can check back on his or her first impression. The subject is asked to assign an order to the sample according to his or her preference. The amount of liking (or disliking) for individual samples cannot be adequately determined by this method.

iii) Rating Scale

Scale ratings reflect panelist's perceived intensities of a specified attribute under a given set of conditions. Various rating scales have been developed and used:

a) Hedonic Rating Scale

This test is used to measure the level of liking for food products by a population. It may be applied in testing for presence or acceptance. The method relies on panelist's capacities to report directly and reliably, their feelings of like and dislike. Several variations of the traditional nine-point hedonic scale have been used effectively. These include:

- Reduced number of rating categories, although not fewer than five is recommended.
- A greater number of "like" rating categories than "dislike",
- Omission of the neutral rating categories by caricatures representing degrees of pleasure and displeasure (facial hedonic scale), and
- Use of non-structured, non-numerical line scale anchored with "like" and "dislike" on opposite ends.

The panelist is asked to evaluate each sample and mark the scales accordingly. Instructions must not influence the panelist's response. Hedonic scale ratings are converted to numerical scores, and statistical analysis is applied to determine difference in degree of liking between or among samples. A hedonic rating test can yield both absolute and relative information about the test samples. Absolute information is derived from the degree of liking or disliking indicated for each sample and relative information is derived from the direction and degree of difference between or among the sample scores.

The data from the hedonic scale ratings are evaluated by rank sum analysis, t-test or chi square test.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the hedonic scale test is as below:

Format 7: Specimen Evaluation Card for-Hedonic Scale

Name: ___________________ **Date:**______________________

Product:__________________ **Time:** _____________________

Test this sample and check appropriate box how much you like or dislike.

Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

Please give your reason for this attitude.

Remember you are the only one who can tell what you like.

An honest expression of your personal feeling will help us.

Code No.

Like extremely	☐
Like very much	☐
Like moderately	☐
Like slightly	☐
Neither like nor dislike	☐
Dislike slightly	☐
Dislike moderately	☐
Dislike very much	☐
Dislike extremely	☐

Comments.

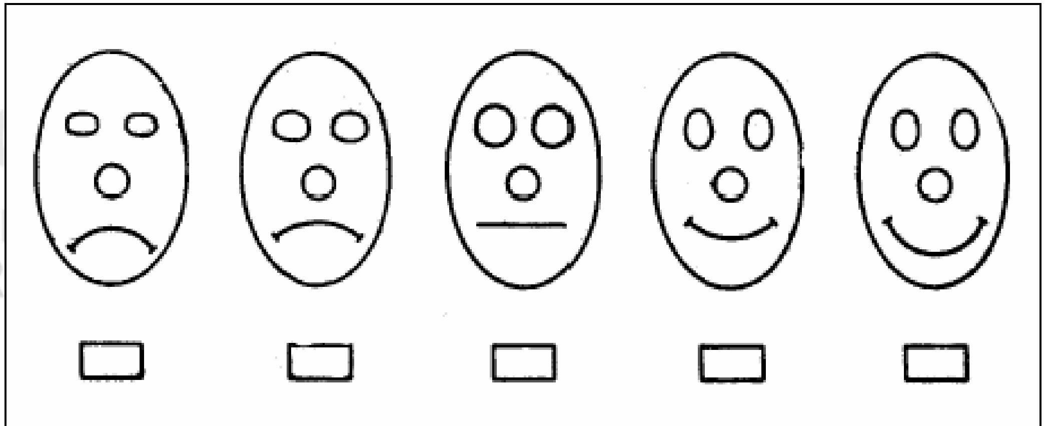
Signature

Format 8: Specimen Evaluation Card for-Hedonic Scale (facial)

Name: ___________________ **Date:**______________________

Product:__________________ **Time:** _____________________

Please check the box under the figure which best describes how you feel about this product.



Signature

b) Food Action Scale Rating

This test may be used to measure the level of acceptance of food products by a population. The scale is not applicable for rating specific characteristics; rather it is a measure of general attitude towards food product. This rating scale includes action as well as affective type statements. Nine successive rating categories ranging from "I would eat this every time it, I have an opportunity" to "I would eat this only if I were forced to" are represented. Samples are presented sequentially in a balanced order, and the panelist is told to decide which of the statements on the scale best represents his or her attitude. Subjects are allowed to make their own inferences about the meaning of the scale categories. The scale ratings are converted to numerical scores to facilitate analysis of data.

The data is evaluated using the evaluation card having the judgement of the panelist. A typical evaluation card for the dilution test is as below:

Let us take another example for sensory evaluation of a product for customer acceptance and preference. For evaluating sensory attributes of a soft drink, following Acceptance Tests can be undertaken:

Taste : Water-like
Strongly sweet
Sweet
Salty
Strongly salty
Medicinal

Viscosity : Less Viscous (e.g. water-like)

Highly viscous (e.g. honey-like)

Consistency : Homogeneous

Heterogeneous

Format 9: Specimen Evaluation Card for-Food Action Scale Rating

Name: ___________________ **Date:**______________________

Product:__________________ **Time:** _____________________

Indicate in appropriate box which of nine statements on the following scale best represent your attitude towards the product.

Code No._______________

I would eat this every opportunity I had	☐
I would eat this very often	☐
I would frequently eat this	☐
I like this and would eat it now and then	☐
I would eat this if available but would not go out of my way	☐
I don’t like it would eat it on an occasion	☐
I would hardly ever eat this	☐
I would eat this only if there were no other food choices	☐
I would eat this only if I were forced to	☐

Comments.

Note — The word ‘eat’ may be replaced by ‘drink’, ‘buy’ or ‘use’.

Signature

14.6 APPLICATIONS OF SENSORY EVALUATION

The sensory evaluation tests are commonly being use in food industry as well as certain other industrial applications as follows:

1) New Product Development

Some new products are unique, but most of them are imitations or variations of some established standards. In either case, the product developer needs information on acceptability to consumers as an input for marketing. The acceptability of products can be evaluated following the given sequence:

a) Characterization of product prototype sample to determine uniqueness.

b) Evaluation of the experimental prototype samples to establish whether differences exist among them.

c) Determination of whether the prototype samples meet the acceptability requirements established for the product.

2) Product Improvement/Process change/cost reduction

Improvement in the products can be judged based on sensory evaluation in following ways:

a) Difference tests to determine whether the experimental product is the same or different from the control.

b) Affective test: If product differs, to establish whether the experimental product is liked more than the control.

3) Quality Control

Representative samples are usually evaluated by difference tests and descriptive tests to ensure that the end product is having all the required qualities during production, distribution and marketing.

4) Storage Stability studies

These are conducted to establish information on product shelf life during transportation, warehousing, retailing and during storage. Representative samples are obtained, evaluated initially and then at specific time intervals of storage. Sensory tests are also used to determine product storage stability such as:

a) Difference tests to determine whether the storage samples are different from the control (if no significant difference is found, product stability is assumed).

b) Descriptive tests used alone or in conjunction with difference tests, to characterize and/or quantify the changes that may have occurred during storage.

c) Acceptance tests to determine the relative acceptance of stored product.

5) Product grading or Rating

This requires an accurate classification of samples according to the grade standards defined for the product; as well as an evaluation of samples in relation to each other. Category scoring or ratio scaling based on the presence and intensity of selected characteristics may be used to measure samples against standard specifications set for the product.

Check Your Progress Exercise 2

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

1) What is required for sensory evaluation test?

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2) Which test measures quantitative characteristics of a product and how?

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3) What is Affective tests and what are the types of affective tests?

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4) What are the different types of rating scales?

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14.7 KEY WORDS

Sensory Analysis	:	Examination of organoleptic attributes of a product by the sense organs.
Sensory (adj.)	:	Relating to the use of the sense organs.
Organoleptlc (adj.)	:	Relating to an attribute of a product perceptible by the sense organs.
Sensation (noun)	:	Subjective reaction resulting from sensory stimulation.
Assessor (sensory) (noun)	:	Any person taking part in a sensory test.
Attribute (noun)	:	Perceptible characteristic.
Acceptability (noun)	:	State of product favourably received by a given individual or population, in terms of its organoleptic attributes.
Acceptance (noun)	:	The act of a given individual or population of finding that a product answers satisfactorily to his/her/its expectations.

Preference (noun)	:	Expression of the emotional state or reaction of an assessor which leads him/her to find one product better than one or several others.
Discrimination (noun)	:	Act or qualitative and/or quantitative differentiation between two or more stimuli.
Hedonic (adj.)	:	Relating to like or dislike.
Quality (noun)	:	Collection of features and characteristics of a product or service that confer its ability to satisfy stated or implied needs.
Product (noun)	:	Edible or inedible matter which can be evaluated by sensory analysis. Examples: food products, cosmetics, textile and fabrics.
Bias (noun)	:	Systematic errors which may be positive or negative.

14.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISE

Check Your Progress Exercise 1

Your answer should include following points:

1) Sensory Evaluation is defined as "*a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, taste, touch and hearing*".

2) Sensory evaluation is normally carried out by designed experiments under proper environmental conditions by both trained and untrained person and they are called sensory panel.

3) There are three types of panels:

   i) Trained Panel (Laboratory Panel)

   ii) Discriminatative and communicative Panel (semitrained Panel)

   iii) Untrained Panel (Cosumer Panel)

Check Your Progress Exercise 2

Your answer should include following points:

1) Selection of the proper panel:

   i) Maintaining suitable environmental conditions and use of standard equipment for the test;

   ii) Obtaining representative samples;

   iii) Preparation and presentation of samples for evaluation in a manner that ensures the uniformity and representation of the samples; and

   iv) Selection of the proper methods and statistical techniques.

2) Descriptive tests using scaling and profiling methods.
3) Affective tests refer to preference testing based on the measurement of preference, or a measure from which relative preference may be determined, e.g. pleasure-displeasure, like-dislike.

   There are three types of affective tests:

   i) Paired – Performance Test

   ii) Ranking Test

   iii) Rating Scale (Hedonic Rating Scale, Food Action Scale Rating)

4) i) Hedonic Rating Scale

   ii) Food Action Scale Rating

14.9 SOME USEFUL BOOKS

Sensory Evaluation Guide for Testing of Food and Beverage Products. By Sensory Evaluation Division, Institute of Food Technologists*; Food Technology, November 1981; 50-59*.

Handbook of Analysis and Quality Control for Fruits and Vegetable Products. S. Ranganna, II Edn. 1994. Tata Mc Graw-Hill Publishing Co. N. Delhi. Chapter 19: Sensory Evaluation.

IS 6273 – 1974 (Reaffirmed 2002): *Guide for Sensory Evaluation of Foods*; *Part I: Optimum Requirements*.

IS 6273 – 1974 (Reaffirmed 2002): *Guide for Sensory Evaluation of Foods*; *Part II: Methods and Evaluation Cards*.

IS 5126 – 1996/ISO 5492 - 1992 (Reaffirmed 2001): *Sensory Analysis – Vocabulary*.

IS 8140 – 1976 (Reaffirmed 2002): *Guide for Selection of Panel for Sensory Evaluation of Foods and Beverages*.