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National Higher School of Agronomy

Department of Food Sciences

Laboratory of Food Technology and Human Nutrition

Course Title:

Analysis of Food Materials

For Study Level:

Master 1 (Food Technology / Food and Human Nutrition)

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Academic Year: 2025-2026

Ecole Nationale Supérieure Agronomique

Département de Technologie alimentaire et Nutrition Humaine

Analysis of Food Materials

Course Title: Analysis of Food Materials

For Study Level: 4th Year in Food Technology / Food and Human Nutrition

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Preface

This course on Analysis of Food Materials is designed to equip students with essential skills and understanding in the field of food analysis, emphasizing both theoretical and practical knowledge. As food safety and quality control become increasingly important in the global market, it is crucial for future professionals in Food Industry Technology and Human Nutrition to master techniques that assess and ensure the safety, nutritional value, and quality of food products. This course will guide students through fundamental analytical processes, including sampling, organoleptic assessment, and the quantitative analysis of various nutritional and potentially toxic elements.

Through hands-on practical sessions, students will directly apply these methods, which will build their competency in handling modern food analysis challenges. This course serves as a foundational component for careers in the food industry, public health, and regulatory agencies.

This course aims to provide students with an in-depth understanding of the methods used to analyze food components, including sampling, organoleptic properties, and the measurement of nutritional and toxic constituents. The students will be able to develope the competencies below

- Mastery in using analytical techniques for assessing food quality and safety.
- Ability to perform precise sampling and apply various measurement methods for food constituents.
- Ability to analyze, interpret, and critically assess food analysis data to make informed decisions.
- Understanding of legislation and regulatory standards in food toxicology and quality control.
- Proficiency in laboratory techniques for the quantitative analysis of macronutrients, minerals, vitamins, and contaminants.
- Experience in performing and interpreting food analysis tests, including enzymatic activities and detection of anti-nutritional factors.

Total Hours: 25 hours (lecture) + 30 hours (practical work)

List of Abbreviations

AACC: American Association of Cereal Chemists

AAS: Atomic absorption spectroscopy

ADI: Acceptable daily intake

AE-HPLC: Anion exchange high performance liquid chromatography

ANFs: Antinutritional factors

AOAC: Association of Official Analytical Chemists

AOCS: American Oil Chemists' Society

ATCC: American Type Culture Collection

ATCC: American Type Culture Collection

BSA: bovine serum albumin

CAA: The Color Additives Amendment

DHHS: Department of Health and Human Services

DSHEA: Dietary Supplement Health and Education

DV: Daily Value

Dwb: dry weight basis

EDTA: Ethylenediaminetetraacetic acid

EEC: European Economic Community

ELISA: Enzyme linked immunosorbent assay

EPA: Environmental Protection Agency

EU: European Union

FAA: The Food Additives Amendment

FAO: Food and Agriculture Organization

FCC: Food Chemicals Codex

FCM: Food contact materials

FD&C: Federal Food, Drug, and Cosmetic

FDA: The Food and Drug Administration

FSANZ: Food Standards Australia New Zealand

GC: Gas chromatography

GC-MS: Gas chromatography – mass spectrometry

GMO: Genetically modified organisms

GMP: Good manufacturing practice

HACCP: Hazard analysis and critical control point

HMs: Heavy metals

HPLC: High-performance liquid chromatography

ISE: Ion-selective electrodes

ISO: International Organization for Standardization

IU: International units

IUPAC: International Union of Pure and Applied Chemistry

JECFA: Expert Committee on Food Additives

Km: Michaelis constant

LC-MS: Liquid chromatography – mass spectroscopy

MRLs: Maximum Residue Limits

MS: Mass spectrometry

NLEA: Nutrition Labeling and Education

NS: Not Specified

RASFF: Rapid Alert System for Food and Feed

RI: Refractive index

SDGs: Sustainable Development Goals

TLC: Thin-layer chromatography

USDA: United States Department of Agriculture

USP: United States Pharmacopeia

Vm: maximum velocity

WHO: World Health Organization

Wwb: wet weight basis

Introduction:

Food is a more basic need of human than that of shelter and clothing. It provides adequately for the body's growth, maintenance, repair, and reproduction. Plant and animal origin are the sources of foods that contain the essential nutrients such as carbohydrates, fats, proteins, vitamins, and minerals. Usually after consumption, food undergoes different metabolic processes that eventually lead to production of energy, maintenance of life, and/or stimulation of growth.

Food analysis is the process for both fresh and processed products by the standardized form and are those most commonly used in the laboratory. These analytical procedures are used to provide information about a wide variety of different characteristics of foods, including their composition, structure, physicochemical properties, phytochemical properties, and sensory attributes.

Investigations in food science and technology, whether by the food industry, governmental agencies, or universities, often require determination of food composition and characteristics. Trends and demands of consumers, the food industry, and national and international regulations challenge food scientists as they work to monitor food composition and to ensure the quality and safety of the food supply. It is harmful for the consumers to consume unsafe foods. A food may be considered unsafe when it contains harmful microorganisms (e.g., Listeria, Salmonella), toxic chemicals (e.g., pesticides, herbicides) or extraneous matter (e.g., glass, wood, metal, insect matter). Therefore, food manufacturers should take all the preventive measures to eliminate these harmful substances from food. Government laboratories use analytical techniques to analyze food and detect toxic substances and also to ensure that the food is safe for consumers.

All food products require analysis as part of a quality management program throughout the development process (including raw ingredients), through production, and after a product is in the market. The characteristics of foods (i.e., chemical composition, physical properties, and sensory properties) are used to answer specific questions for regulatory purposes and typical quality control. The nature of the sample and the specific reason for the analysis commonly dictate the choice of analytical methods. Speed, precision, accuracy, and ruggedness often are key factors in this choice.

Validation of the method for the specific food matrix being analyzed is necessary to ensure usefulness of the method. Making an appropriate choice of the analytical technique for

a specific application requires a good knowledge of the various techniques. For example, your choice of method to determine the salt content of potato chips would be different if it is for nutrition labeling than for quality control. The success of any analytical method relies on the proper selection and preparation of the food sample, carefully performing the analysis, and doing the appropriate calculations and interpretation of the data. Methods of analysis developed and endorsed by several nonprofit scientific organizations allow for standardized comparisons of results between different laboratories and for evaluation of less standard procedures. Such official methods are critical in the analysis of foods, to ensure that they meet the legal requirements established by governmental agencies.

The most important element and ultimate goal in food quality control is protecting the consumer. To ensure standardization of these procedures, food laws and regulations cover the related acts affecting the marketing, production, labeling, food additive used, dietary supplements, enforcement of **good manufacturing practice (GMP), hazard analysis and critical control point (HACCP),** federal laws and regulations, factory inspections, and import/export inspections.

This module deals with the principles of these different analytical methods of plant or animal origin and parameters that are essential in food analysis and quality control. The targets of this course are:

1. to help students to understand the principles behind analytical techniques associated with food,
2. to help students to being able to select the appropriate analytical technique when presented with a practical problem, and,
3. to help students demonstrating practical proficiency in food analysis laboratory.

Part I.

1. Purpose of food analysis

Food control and inspection demand particular attention, especially because animals and fish can be affected by numerous diseases, which can be transmitted to humans. Moreover, the street food sector is significant, informal, and complex. It represents a livelihood and a source of accessible and affordable food for millions of people. Additionally, the ingredients used in these foods are not subjected to appropriate controls. Consequently, all such foods sold on the streets can pose serious risks of food poisoning, through microbiological contamination, improper use of food additives, or the presence of environmental contaminants residues. Inspection activities must cover the entire sequence "from farm to fork" to ensure no health risks along the food chain, aiming to:

- Preserve the quality and safety of food products at every stage;
- Identify any potential damage during transportation;
- Maintain consumer confidence and brand reputation;
- Avoid safety-related recalls;
- Comply with regulations and standards of destination markets.

1.1 Nutritional labelling:

Consumers have many choices regarding their food supply, so they can be very selective about the products they purchase. They demand a wide variety of products that are of high quality, nutritious, and offer a good value. Also, consumers are concerned about the safety of foods, which has increased the testing of foods for allergens, pesticide residues, and products from genetic modification of food materials. Many consumers are interested in the relationship between diet and health, so they utilize nutrient content and health claim information from food labels to make purchase choices. These factors create a challenge for the food industry and for its employees.

Nutritional assessment of foods is the best way to determine whether nutritional needs are effectively met and if the biochemical composition of foods truly conforms to standards.

1.2 Hygienic Perspective

Food hygiene legislation establishes the basic principles and rules that food establishment owners and operators must adhere to during the preparation, processing, manufacturing, handling, packaging, transport, storage, and distribution of food, to ensure a safe, good quality product fit for human consumption. To compete in the marketplace, food companies must produce foods that meet the demands of consumers.

1.3 Legal Aspect:

To market safe, high-quality foods effectively in a national and global marketplace, food companies must pay increasing attention to government regulations and guidelines and to the policies and standards of international organizations. Food scientists must be aware of these regulations, guidelines, and policies related to food safety and quality and must know the implications for food analysis.

1.3.1 International standards and policies :

With the need to compete in the worldwide market, employees of food companies must be aware that allowed food ingredients, names of food ingredients, required and allowed label information, and standards for foods and food ingredients differ between countries. To develop foods for, and market foods in, a global economy, one must seek such information from international organizations and from organizations in specific regions and countries.

1.3.2 Codex Alimentarius

The Codex Alimentarius Commission (Codex Alimentarius is Latin for "code concerned with nourishment") was established in 1962 by two United Nations organizations, the **Food and Agriculture Organization (FAO)** and the **World Health Organization (WHO)**, to develop international standards and safety practices for foods and agricultural products. The standards, published in the Codex Alimentarius, are intended to protect consumers' health, ensure fair business practices in food trade, and facilitate international trade of foods.

1.3.3 The Food and Drug Administration (FDA)

FDA is a government agency (USA) within the Department of Health and Human Services (DHHS). The FDA is responsible for regulating, among other things, the safety of foods, cosmetics, drugs, medical devices, biologicals, and radiological products. It acts under laws passed by the US Congress to monitor the affected industries and ensure the consumer of the safety of such products.

1.3.4 The Hazard Analysis Critical Control Point (HACCP)

The HACCP concept has been adopted not only by the US Food and Drug Administration (FDA) and other federal agencies in the USA, but also by the Codex Alimentarius Commission, an international organization that has become a major force in world food trade. HACCP is an internationally recognized systematic approach that is used to prevent and/or control microbial, chemical, and physical hazards within the food supply. The "farm to the fork" approach was originally designed to be used by the food processing

industry to produce zero defect (no hazard) food for astronauts to consume on space flights. The HACCP program approach is based on seven principles identified below:

1. Determine potential microbial, chemical, and physical hazards in each step of the process flow.
2. Identify critical control points in the process.
3. Establish control limits for each critical control point.
4. Establish procedures to monitor control points.
5. Establish corrective actions when limits of control point are exceeded.
6. Establish appropriate system of record keeping.
7. Establish program to verify and validate efficacy of program.

1.3.5 ISO Standards:

In addition to food standards and policies established by the Codex Alimentarius Commission, **the International Organization for Standardization (ISO)** has the 9000 series of standards on quality management and quality performance. The intent of the quality management standards is to establish a quality system, maintain product integrity, and satisfy customers. ISO 9001:2000 focuses on a process approach to quality management.

1.3.6 Anothers standards:

Other international, regional, and country-specific organizations publish standards relevant to food composition and analysis. For example, the **Saudi Arabian Standards Organization (SASO)** publishes standards documents (e.g., labeling, testing methods) important in the Middle East (except Israel), and the European Commission sets standards for foods and food additives for countries in the **European Economic Community (EEC)**. In the USA, the Food Ingredients Expert Committee, which operates as part of the US Pharmacopeia, sets standards for the identification and purity of food additives and chemicals, published as the **Food Chemicals Codex (FCC)**. For example, a company may specify in the purchase of a specific food ingredient that it be "FCC grade." Countries other than the USA adopt FCC standards (e.g., Australia, Canada).

Part II.

2. Preliminary operations for food control

The raw materials for the food industry are usually collected or harvested in farms and open land, and the means of transporting them to the processing plants take many forms. Raw materials arriving at processing factories, therefore, exposed to various contamination sources, so the logical order of preliminary operations is cleaning, sorting, and grading. Transporting of raw materials includes such varied operations as hand and mechanical harvesting on the farm, refrigerated trucking of perishable produce, boxcar transportation of live cattle, and pneumatic conveying of flour from a rail car to bakery storage bins. Throughout such operations, emphasis must be given to maintaining sanitary conditions, avoiding product losses, maintaining raw material quality, minimizing bacterial growth, and timing all transfers and deliveries so as to shorten the holdup time, which can be costly as well as detrimental to product quality.

2.1.Sampling

Sampling purposes vary widely among different food industries; however, the most important categories include the following:

- Nutritional labeling.
- Detection of contaminants and foreign matte.
- Statistical process control (Quality Assurance).
- Acceptance of raw materials, ingredients, or products (Acceptance Sampling).
- Release of lots of finished product.
- Detection of adulterations.
- Microbiological safety.
- Authenticity of food ingredients, etc.

The major steps in sampling are:

- Identification of the population from which the sample is to be obtained,
- Selection and obtaining of gross samples of the population, and.
- Reduction of each gross sample to a laboratory-size sample suitable for analysis.

The **International Union of Pure and Applied Chemistry (IUPAC)** defines a sampling plan as "A predetermined procedure for the selection, with-drawal, 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 goals of the sampling plan, the factors to be measured, sampling point, sampling procedure, frequency, size,

personnel, preservation of the samples, etc. The primary aim of sampling is to obtain a sample, subject to constraints of size that will satisfy the sampling plan specifications.

Sampling plans are composed of three components:

i. Sampling,
ii. Sample preparation, and.
iii. Analysis.

2.1.1 Simpling techniques

Foodstuffs are relatively heterogeneous materials, so sampling and any subsequent separation are the greatest source of error in food analysis. The problem may be minimized by selecting either randomly or according to a plan, several samples from the lot. In sampling foods and food products, sufficient material must be taken to compensate for the variability involved. The number of individual samples to be selected may be calculated from the following expression:

$$n = C\sqrt{N}$$

Where,

n= number of individuals to be selected.

C= is a factor which represents the degree of accuracy desired in the sample, and.

N= lot size.

The sample selected should be representative, and reflect all the homogeneous parts of the heterogeneous population. Generally, the errors in sampling are due to:

- Lack of randomness in selection.
- Change in composition of product during sampling.
- Non-homogeneity of food.

2.1.2 Sample

The Analytical Commission of Terminology of IUPAC (International Union of Pure and Applied Chemists) has proposed the definition of sample as "A portion of material taken from the consignment and selected in such a way that it possesses the essential characteristics of the bulk".

An ideal sample should be identical in all its intrinsic properties with the bulk of the material from which it is taken. The sample should be large enough for all intended determination. Homogenous samples of 250 g are generally sufficient. The size of the sample varies from product or material to material and type of analysis e.g. samples of spices are often limited to 100 g and of fruits or vegetables increased to 1000 g. The sample should be

packed and stored in such a way that no significant change occur from the moment of sampling until the analysis is complete.**Table. 1** present the glossary of sampling terms.

Table 1: Glossary of sampling terms

Term	Definition
Sample	A portion of a population or lot; may consist of an individual or groups of individuals.
Sub Sample	A portion taken from a sample; a laboratory sample may be a sub sample of a gross sample; similarly, a test portion may be a sub sample of a laboratory sample.
Gross Sample	Also called bulk sample, lot sample; one or more increments of material taken from a larger quantity (lot) of material for assay or record purposes.
Composite Sample	A sample composed of two or more increments.
Laboratory Sample	A sample, intended for testing or analysis, prepared from a gross sample or otherwise obtained; the laboratory sample must retain the composition of the gross sample; often reduction in particle size is necessary in the course of reducing the Quantity.
Test Portion	Also called specimen, test specimen, test unit, aliquot; that quantity of a material of proper size for measurement of the property of interest; test portions may be taken from the gross sample directly, but often preliminary operations, such as mixing or further reduction in particle size, are necessary.
Segment	A specifically demarked portion of a lot, either actual or hypothetical.
Strata	Segments of a lot that may vary with respect to the property understudy.
Population	A generic term denoting any finite or infinite things, objects, or events in the broadest concept; an aggregate determined by some property that distinguishes things that do and do not belong.
Lot	A quantity of bulk material of similar composition whose properties are under study.
Increment	An individual portion of material collected by a single operation of a sampling device, from parts of a lot separated in time or space; increments may be either tested individually or combined (composited) and tested as a unit.
Individual	Conceivable constituent part of the population.
Bulk Sampling	Sampling of a material that does not consist of discrete, identifiable, constant units, but rather of arbitrary, irregular units.
Homogeneity	The degree to which a property or substance is randomly distributed throughout a material; homogeneity depends on the size of the units under consideration; thus a mixture of two minerals may be inhomogeneous at the molecular or atomic level, but homogeneous at the particulate level.
Reduction	The process of preparing one or more sub samples from a sample.

2.1.3 Preparation of Samples

2.1.3.1 General size reduction considerations

If the particle size or mass of the sample is too large for analysis, it must be reduced in bulk or particle size **(Fig.1)**. To obtain a smaller quantity for analysis the sample can be spread on a clean surface and divided into quarters.

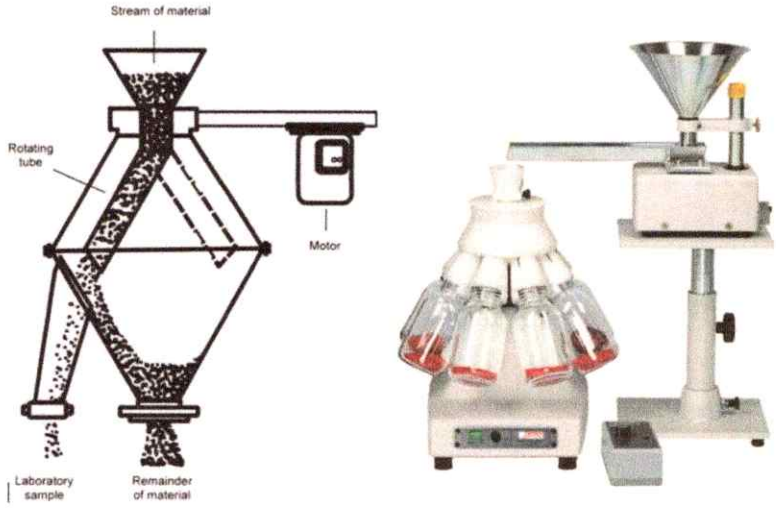


Figure.1 (Left) A rotating tube divider for reducing a large sample (ca. 880 kg) of dry, free-flowing material to a laboratory size sample (ca. 0.2 kg). (Right) The Rotary Sample Divider Laborette 27 splits fine-powdered.

In order to obtain precise analytical results, the laboratory sample must be made as homogeneous as possible so that, within the limits of analytical method used, the replicate analyses agree as closely as possible. The purpose of sample preparation is to mix thoroughly a large sample in the laboratory. This apparently homogenous sample must be then reduced in size and amount for subsequent analysis. The problems encountered by the analysts in the preparation of samples for analysis include:

- Preparing representative small samples from large samples,
- Loss of plant material,
- Removal of extraneous material from plants without removal of plant constituents,
- Enzymatic changes before and during analysis,
- Compositional changes during grinding, vi metal contamination during grinding,
- Changes in unstable components, and,
- Special preparation problems in analysis of oilseed materials.

The sample to be prepared should be first homogenized and the method of homogenization will depend on the type of food being analyzed. A number of very efficient electrical mechanical devices are available to reduce the size of food particles and to mix food products thoroughly. Mincers, graters, blenders and homogenizers (for dry, moist and wet foods) and various types of powder mills or grinders are essential equipment in a food laboratory. Both the nature of food material and the analysis to be performed must be considered in the selection of instrument for grinding.

2.1.3.2 Preparation of dry food samples

For grinding of dry materials, mechanical methods range from the simple pestle and mortar to elaborate and effective devices for grinding. For fine grinding of dry materials, power-driven hammer mills are widely used. Hammer mills are used to grind such materials as cereals, oil meals and most foods, which are reasonably dry and do not contain excessively high amounts of oil or fat. Grinding of oil seeds or oil rich samples present special problems. Dried fruits should be passed through chopper three times and mixed thoroughly. If needed, initially, grinding can be done by coarse cutting blade.

2.1.3.3 Preparation of moist solid foods

Moist solid foods such as meat products are best homogenized by chopping rather than mincing. Cheese and chocolates are best grated followed by hand mixing of the, rated material.

For disintegrations of moist materials various fine-slicing devices are available. Bowl cutters (leafy vegetables, fleshy tubers and roots) or meat mincers (fruits, roots and meat products) disintegrate some moist materials best. Chilled ball mills can be used to grind frozen materials without preliminary grinding. Grinding of frozen foods reduces undesirable chemical changes. The commercially available tissue grinders are also used for small sample of soft material.

For preparation of sample of fresh fruits and vegetables, first of all it is essential to remove adhering soil or sand by washing or wiping with damp cloth. Excessive washing should be avoided to prevent leaching of soluble solids. Then, separate the fresh tissues into core, outer and inner tissue depending on the objectives of analysis.

2.1.3.4 Preparation of semi-solid/liquid foods

Fluid foods are best emulsified by top or bottom driven blenders. Fruit juice beverages containing insoluble matter, should be blended using high-speed blender to get uniform sample. Pureed products such as tomato puree, ketchup, fruit pulps and strained fruits and vegetables should be thoroughly shaken before sampling.

Gentle warming and mixing easily prepare oils and fats. Butter and margarine may be re-emulsified by shaking by hand in a glass jar after warming to 35 °C to melt the fat.

2.1.3.5 Enzyme inactivation

Enzyme naturally present may cause undesirable changes during preparation of samples for analysis. Generally, if total contents of a specified compound are determined i.e. minerals, carbohydrates, nitrogen, enzyme inactivation is not essential. However, if sugars, free and bound forms of lipids, groups of proteins are to be determined, the tissues must be killed in such a way that potentially troublesome enzymes are immediately and completely inactivated.

To preserve the original state of components in living tissues, several methods of enzyme inactivation can be used. The treatment required for enzyme inactivation varies widely with the food size, consistency, composition, and the enzyme present and intended analytical determinations.

Enzymes may be inactivated with steam or boiling alcohol. Fungal amylases are generally heat labile and can be inactivated at relatively low temperatures; some bacterial amylases are highly heat resistant. Extraction of chlorogenic acid from seed or dry tissues requires heating to 90- 100 °C for 1 hr. to inactivate polyphenolases.Some enzymes can be inactivated by inorganic compounds that cause irreversibly enzyme poisoning, by a shift in pH, or by salting out. The most common method of inactivating enzyme include treatment with 80 % methanol or ethanol, ice-cold 5- 10 % perchloric or tri-chloroacetic acid or a mixture of methanol- chloroform- 2M formic acid (12:5:3 by volume).

2.1.3.6 Lipid Oxidation Protection

Lipids present particular problems in sample preparation. High-fat foods are difficult to grind and may need to be ground while frozen. Unsaturated lipids are sensitive to oxidative degradation and should be protected by storing under nitrogen or vacuum. Antioxidants may stabilize lipids and may be used if they do not interfere with the analysis. Light- initiated photo oxidation of unsaturated lipids can be avoided by controlling storage conditions. In practice, lipids are more stable when frozen in intact tissues rather than as extracts (Cubadda

et al., 2001). Therefore, ideally, unsaturated lipids should be extracted just prior to analysis. Low-temperature storage is generally recommended to protect most foods.

2.1.3.7 Microbial Growth and Contamination

Microorganisms are present in almost all foods and can alter the sample composition. Likewise, microorganisms are present on all but sterilized surfaces, so sample cross-contamination can occur if samples are not handled carefully. The former is always a problem, and the latter is particularly important in samples for microbiological examination. Freezing, drying, and chemical preservatives are effective controls and often a combination of these is used. The preservation methods used are determined by the probability of contamination, the storage conditions, storage time, and the analysis to be performed (Cubadda et al., 2001).

2.1.4 Sampling Types by Process

Samples for analysis should be large enough for all intended determinations. Homogeneous samples of 250 g (or ml) are generally sufficient. Samples of spices are often limited to 100 g, and those of fruits and vegetables increased to 1000 g. Samples should be packed and stored in such a way that no significant changes occur from the moment of sampling until the analysis is completed. The container should be identified clearly. Official and legal samples must be sealed in such a. way that they cannot be opened without breaking the seal.

2.1.4.1 Manual Sampling

Samples are frequently taken manually. Apparently homogeneous materials such-as single-phase liquids or well-mixed powders should be mixed thoroughly immediately before sampling. Rotating and shaking in a closed container that has a volume at least twice that of the sample can mix small quantities of powders or solutions.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. Probes and triers generally sample granular or powdered solids.

2.1.4.2 Continuous sampling

Continuous sampling is performed mechanically. Continuous sampling should be less prone to human bias than manual sampling.

2.1.5 Homogeneous vs. 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.

2.1.6 Storage and Preservation of Samples

Prepared samples may undergo changes in composition through evaporation or absorption of moisture or by the action of enzymes or microorganisms. The components that are likely to change e.g. ascorbic acidshould be analyzed immediately after preparation in fresh material. Products that are likely to undergo microbial spoilage may be preserved by using preservatives, by freezing, or by drying.

Sample containing moisture can be dried as rapid and at as low a temperature possible. Spreading the sample over a wide area can facilitate drying. Generally, drying at 60 °C under vacuum is recommended. If the sample contain no heat-sensitive or volatile compounds, heating for several minutes at 70 to 80 °C may be advisable. Such heating also inactivates most enzymes. During drying, certain components are destroyed (enzymes, vitamins), other are almost invariably modified (proteins and lipids) and some flavor components are volatilized. If drying is not done carefully, caramelization and sugar inversion in acid foods are likely to occur.

Some plant materials can be stored at -20 to -30 °C provided they can be cooled to low temperature within 1 h. Freezing of samples in air and moisture proof containers by rapid freezing and storage at less than -6.7 °C prevents microbial activity but not the enzyme activity which continues to occur at temperature down to -40 °C, although it a slower rate. Most foods are preserved best by freeze-drying. Fresh foods, in which enzymes have not been inactivated prior to freezing, are especially susceptible enzymatic attack during and after thawing. Sample rich in lipids must be chilled rapidly prior to extraction or frozen quickly for storage. Addition of antioxidants (0.1- 0.05 % of Propyl gallate or santoquin) is effective provided it does not interfere with the analytical determinations. Polyunsaturated fatty acid-,

are less damaged when stored in frozen (-20 °C) intact tissues than after they are extracted from the tissues.

Storage of dried products at 0 to 10 °C minimizes deterioration. To reduce or eliminate microbial attack, preservatives such as sorbic acid, sodium benzoate, sodium salicylate, tyrosin, formaldehyde, mercuric chloride, toluene, or thymol are used. Selection of preservatives, however, will depend upon the nature of food, expected contamination, storage period and analysis to be performed. All prepared food samples should be rapidly transferred to dry glass or plastic containers with well-fitted lid, clearly labeled and stored at a suitable low temperature.

2.1.7 Sampling Errors

Sampling errors are caused by several factors.

- Lack of randomness in sample selection may result from either instrumental limitations or deficiencies and from human bias.
- Manual methods of sampling powdered or granular materials are subject to numerous errors. Most of these difficulties are overcome by fine grinding and mixing of large samples.
- Changes in composition may occur during or after sampling. Typical changes include gain or loss of water, loss of volatiles, physical inclusion of gases, reaction with container material or foreign matter in container, and damage to fruits or vegetables by mechanical injury leading to enhanced enzymatic or chemical changes.

2.2 Sensory Evaluation for Food Quality Assessment

Quality is the ultimate criterion of the desirability of any food product. Food quality can be evaluated by sensory and objective method.

2.2.1 Sensory Evaluation

"Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of food and materials as they are perceived by the senses of sight, smell, taste, touch and hearing". IFT 1975 **(Fiszman et al., 2015)**.

When the quality of a food product is assessed by means of human sensory organs, the evaluation is said to be sensory, subjective, or organoleptic. Every time food is eaten, a judgement is made. Sensory quality is a combination of different senses of perception coming into play in choosing and eating a food. Appearance, flavor and mouthfeel decide the

acceptance of the food. This reaction is highly conditioned by a variety of psychological and social factors and in the final analysis, plays a vital role in the acceptance and preference of foods.

2.2.2 Sensory Characteristics of Food

2.2.2.1 Appearance

Surface characteristics of food products contribute to the appearance. Scrambled egg with a very dry surface is not acceptable. Fudge with a glossy surface is rated high. Interior appearance can also be evaluated. The eye can judge lumps in a pudding or gravy that are not desirable. Sight plays a role in the assessment of the lightness of foods like the bread, cakes and idli. Keep this perception of the size, shape of the foods and of such characteristics as transparency, opaqueness, turbidity, dullness and gloss is mediated by the organs of sight. Quality of fish can be ascertained by the brightness of the eyes of fish. Quality of sweet limes can be found out by appearance. If the skin is thin, it is juicier. Infestation with insects can be found out in brinjal by the appearance of black spots on it. Completeness of cooking can be judged by appearance in products like meat and rice.

2.2.2.2 Color

In addition to giving pleasure, the color of food is associated with other at- tributes. Ripeness of fruits like banana, tomato, mango, guava, papaya and plum can be assessed by the color. Color is used as an index to the quality of a number of foods. The strength of coffee and tea is judged in part by the color of the beverages. The color of roast beef is used as an index to doneness. Toast, dose, and chapatti which are too brown are likely to be rejected in anticipation of scorched bitter taste.

2.2.2.3 Flavor

The flavor of food has three components-odor, taste and a composite of sensations known as mouth feel.

2.2.2.4 Odor

The odor of food contributes immeasurably to the pleasure of eating. A substance that produces odor must be volatile and the molecules of the substance must be exposed to receptors in the epithelium of the olfactory organ. It is estimated that the olfactory sense of man has the capacity to distinguish 16 million odors. Aroma is able to penetrate even beyond the visual range when comparatively volatile compounds are abundant as is true in boiling sambar. The volatility of aromas is related to the temperature of the food. High temperatures

tend to volatilize aromatic compounds, making them quite apparent for judging; cool or cold temperatures inhibit volatilization.

2.2.2.5 Taste

We value food for its taste. Taste sensation which the taste buds register are categorized as sweet, salt, sour or bitter. Taste buds in the different areas of the tongue are not equally sensitive to all taste stimuli and at least some taste cells respond to more than one stimulus. Taste buds near the tip of the tongue are more sensitive to sweet and salt. Those on the sides to sour and those near the back to bitter. The sensation known as sour is associated with hydrogen ions supplied by acids like vinegar and by those found in fruits and vegetables. Salt taste is due to ions of salt. Sodium chloride is said to be the only one with a pure salt sensation. Substances that elicit the sweet sensation are primarily organic compounds like alcohols, certain amino acids, and aldehydes. Glycerol tastes mildly sweet. Sugars are the main source of sweetness in food. Not all sugars are equally sweet. Fructose gives the most intense sweet sensation followed by sucrose, glucose, maltose, galactose and lactose. Sweetness appears to be associated with the hydroxyl radicals on the sugar molecules. The concentration required for identification is known as the "threshold" for that particular substance. Individuals differ in their sensitivity to the four taste sensations and the threshold for each of the primary tastes is usually not at the same level in any one individual. The pleasant sensations in eating come more from odor than from taste.

a- Taste interaction

Foods contain mixture of substances that elicit all four taste sensations. Salt in sub threshold concentration reduces the tartness of acid. Some threshold concentrations of salt also increase the apparent sweet- ness of sucrose. The addition of salt to lime juice, sherbet, lassi, and to fruits like apple or guava improve the taste. Conversely, acids in sub threshold concentration intensify the saltiness of sodium chloride so it is easy to over salt tart foods. Sugar in sub threshold concentration reduces the saltiness of sodium chloride so a pinch of sugar may improve vegetable soup that has been over salted. Sugar also reduces the sourness of acids and the bitterness of coffee.

b- Mouth feel

Texture and consistency and hotness or burning sensation of pepper can be felt in the mouth.

c- Temperature

Hot and cold sensations contribute to the composite flavor of a food like coffee, soup or ice cream. Taste sensations are less intense as the temperature of food is lowered below 20 °C and raised above 30 °C. Thus, hot coffee is not as bitter as that which has cooled in the cup, iced coffee is not as bitter as that which is warm but not hot. Melted ice cream tastes unpleasantly sweet although in the frozen state it is acceptable.

d- Texture

Texture in ice cream depends upon the size of the crystals. How they feel on the tongue is characterized as coarse or fine. Coarse textured crystalline products are said to be grainy. The brittleness of food is another aspect of texture. Tissues in a raw vegetable and fruit are brittle or crunchy. The cells offer moderate resistance to fraction by the pressure of the teeth e.g. crispness of apple and raw carrots. Tenderness in fruits and vegetables depends on how easily the cells separate. In meats, ease of separation of the lean (without fat) tissue determines the tenderness. Tenderness in pastry is assessed by the ease with which the crisp crust breaks.

e- Astringency

It is dry puckery sensation believed to be due to precipitation of the proteins in the saliva and in the mucous membrane lining of the mouth, which deprives them of their lubricating character. Astringent substances may also constrict the ducts leading from the salivary glands to the mouth. Unripe fruits like cashew fruit, wood apple, blue berry and gooseberry are astringent.

f- Consistency

Ice creams may be too hard or too soft which can be found out by mouth feel. Gravies, sauces and syrups range in consistency from thick to thin. Temperature may affect the consistency of food e.g. ghee, butter, cheese and ice creams. The consistency of soft custard besides being thick or thin may be smooth or curdled. Cream soups may be smooth or lumpy. Gels may be rubbery or fragile (easily breakable). Particles of cooked cereal can be pasty or separate in grains.

g- Psychological factors

In addition to colour, odour, taste and mouth feet, certain psychological factors contribute to the acceptability of foods. Food is accepted when there is pleasant association.

2.2.3 Conducting Sensory Tests

Sensory tests are well integrated with the overall plan of development of the product.

2.2.3.1 Trained panel members

The sensory qualities, particularly the flavor attributes are essentially to be measured subjectively. From early times, this judging has been the preserve of experts who used to evaluate tea, coffee and wine. With the development of sensory evaluation techniques on scientific lines, panels whose sensitivity and consistency have been established by training and repeated tests are replacing the experts. The panel members analyze food products through properly planned experiments and their judgements are quantified by appropriate statistical analysis.

2.2.3.2 Selection of panel of judges

Actually one extremely discriminating pains taking and unbiased individual would suffice for tasting. Further one individual may not be able to discriminate different aspects of food quality. Hence a panel of Judges may be used. Members of the panel should be carefully selected and trained to find out difference in specific quality characteristics between different stimuli and also direction and intensity of difference.

The requirements for an ideal panel member are as follows:

(i) He should be able to discriminate easily between samples and should be able to distinguish appreciable differences in taste and smell.

(ii) He should have good health. If he is suffering from cold his sensitivity may be affected. A sick patient cannot judge the food correctly.

(iii) He should be experienced in the particular field.

(iv) He should have high personal integrity. He should not be prejudiced. He should be able to evaluate objectively.

(v) Willingness to spend time for the sensory evaluation work is required.

(vi) He should have interest in sensory analysis of samples and intellectual curiosity.

(vii) He should have ability to concentrate and derive proper conclusion.

(viii) He should be available and willing to submit to periodic test to get consistent results.

Candidates possessing these qualities must be indexed with details of age, sex, specific likes and dislikes availability. There are different types of panels:

a. Trained panel

Laboratory panels must then be carefully trained for specific products or purposes. These tests aim at finding differences in specific quality characteristics between different stimuli and also direction and/or intensity of the difference. Periodically the panel is given refresher training and tests. The number of members in the trained panel should be small varying from five to 10.

b. Discriminative, communicative or send trained panels

These panels are constituted of technical people and their families, who are normally familiar with the qualities of different types of food. They are capable, with few preliminary test runs, of following instructions for tests given, discriminating differences and communicating their reactions. Such panels of 25-30 are used to find the acceptability or preference of final experimental products prior to large-scale consumer trials.

c. Consumer panels

Such panels are made up of untrained people chosen at random to represent a cross-section of the population for which the product is intended. The greater the number the greater the dependability of the result. A group of not less than 100 is considered the minimum.

2.2.3.3 Testing laboratory

Testing laboratory consists of three separate units.

a. Reception room where the panel members meet the person in charge of the laboratory and are acquainted with the type of the samples to be tested.

b. Sample preparation room that is clean and well equipped for the preparation and serving of samples.

c. Test booths, are where the panel members carry out the actual sensory evaluation of the samples.

- The entire testing laboratory should be air-conditioned, free from noise and extraneous odors.
- Whenever samples with difference in colors are tested, color lights should be used to mask the color of the samples.
- Stainless steel, glass, dishes, cups, and plain serving china are the most convenient as utensils.

2.2.3.4 Preparation of samples

Samples for presentation must be from homogeneous lot. Careful sampling of the food is necessary for sensory evaluation. Samples to be tested should be prepared by identical methods. All samples should be at the same temperature, optimum level and kept constant during the test. Stainless steel forks and spoons can be used for tasting the samples.

2.2.3.5 Techniques of smelling and tasting

For odor tests of food products, a special technique is used to perceive the aroma more clearly. Smelling is done with short, rapid sequence of sniffs. Tasting of coffee or tea or fruit juice is done by slurping. One teaspoon of the liquid is rolled on the tongue so that the liquid reaches all parts of the tongue where the taste buds are located.

2.2.3.6 Tasting time

Tasting should be done at a time when the panel members are fresh. The test time is generally between 10 to 12 in the morning. Too many samples should not be given as they may produce fatigue and lead to errors in the results (Not more than 4-5 samples at a time).

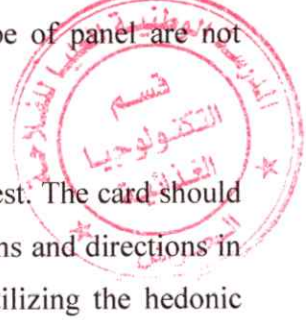
2.2.3.7 Design of expriment

Experimental error can be minimized through the use of techniques of randomizing. A statistical design is used in order to measure variables separately and together and to establish the significance of results. The experiment should be designed based on the accuracy needed and the amount of sample available.

2.2.3.8 Reasons for testing food quality

Two principles reasons:

1. To know the consumer preference. This helps the producer to discover which qualities of the product need to be developed and emphasized.
2. To know the effect of variation in processing on quality Tests are done to investigate the influence of factors in production. They should have the ability to distinguish among degrees of difference in flavor. The members of this type of panel are not required to be expert tasters of the product under investigation.



2.2.3.9 Evaluation Card

The questionnaire or score card should be prepared carefully for each test. The card should be clearly typed or printed. It should be simple and use unambiguous terms and directions in the desired sequence of action as a guide to the evaluation. A table utilizing the hedonic ratings ranging from unacceptable to very acceptable is relatively easy to construct **(Fig.2)**. All scorecards should contain the date and name of the judge.

Scorecard - Hedonic Rating Scale

Tray number ..... Name .....

In front of you is one sample. Taste the sample and tick ✓ howmuch you like or dislike each of the characteristics. You can taste the sample more than once.

	Appearance	Aroma	Taste	Sweetness	Texture/mouth-feel
Like a lot	____	____	____	____	____
Like a little	____	____	____	____	____
Neither like nor dislike	____	____	____	____	____
Dislike a little	____	____	____	____	____
Dislike a lot	____	____	____	____	____

Figure.2 Scorecard used for hedonic rating test (Singh &Maharaj., 2014).

To calculate the score for each product each descriptor was assigned a score value: like a lot = 5, like a little = 4, neither like nor dislike = 3, dislike a little = 2, dislike a lot = 1.

Figure.3 below gives some of the calculations that was done using the data collected.

Record Sheet - Hedonic Rating Scale

Food Characteristic – Appearance, Aroma, Taste, Sweetness, Texure/Mouth-feel

Score Value Assigned:
like a lot = 5 like a little = 4 neither like nor dislike = 3 dislike a little = 2 dislike a lot = 1

	Tester 1	2	3	4	20	Total Score	Average Score (total score ÷ number of testers)
Appearance	5 pts	4 pts	4 pts	4 pts	4 pts	84	4.2
Aroma	4 pts	4 pts	5 pts	5 pts	5 pts	92	4.6
Taste	4 pt	5 pt	5 pts	5 pts	5 pt	96	4.8
Sweetness	2 pt	2 pt	4 pt	4 pt	4 pt	64	3.2
Texture/ Mouth-feel	3 pt	3 pt	2 pt	2 pt	3 pt	52	2.6

Figure.3 Summary of Results from Hedonic Rating Test (Singh &Maharaj., 2014).

❖ **Example: Sensory Evaluation for Fruity Granola Bar (Singh &Maharaj., 2014).**

Hedonic Rating Test and Questionnaire for First Phase, see **(Fig. 4)** for sample of scorecard that was presented. Sensory tests were carried out in a sensory evaluation room, with white light, controlled ventilation, and away from distractions noise, odors and the preparation room. In all phases participants signed an informed consent form and 20

participants were chosen for the pilot testing. Hedonic Rating Test and Questionnaire for First Phase, see **Fig. 4.a** and b for sample of scorecard that was presented and Summary of Results from Hedonic Rating Test respectively.

Scorecard - Hedonic Rating Scale

Tray number ..... Name .....

In front of you is a coded sample. Taste the sample and tick ✓ how much you like or dislike it. You can taste the sample more than once.

	Appearance/colour	Taste/Flavour	Smell/Odour	Texture/Mouthfeel
like extremely				
like very much				
like moderately				
like slightly				
neither like nor dislike				
dislike slightly				
dislike moderately				
dislike very much				
dislike extremely				

(a)

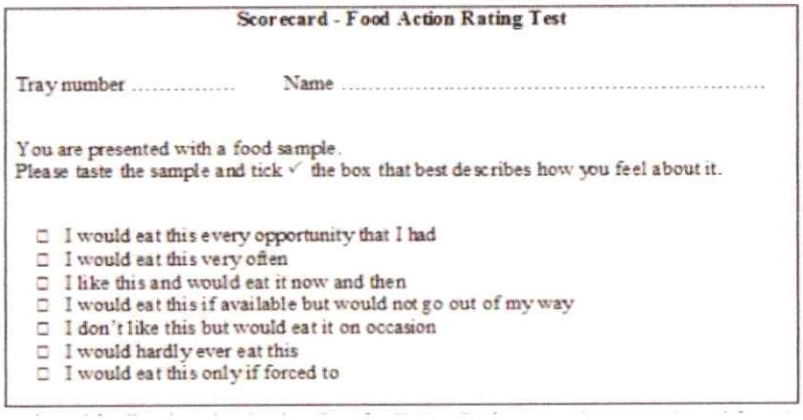
Record Sheet
Hedonic Rating Scale

Food Characteristics - Appearance/colour, Taste/Flavour, Smell/Odour, Texture/Mouthfeel, Sweetness

Score Value Assigned:
Liked extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1

	Tester					Total Score	Average Score (total score ÷ number of testers)
	1	2	3	4	20		
Appearance (colour, shape)	9	9	9	8	7	165	8.3
Taste/Flavour	9	8	7	5	4	148	7.4
Smell/Odour	9	7	8	6	6	152	7.6
Texture/ Mouthfeel	4	7	7	5	6	124	6.2
Sweetness	7	7	8	8	6	150	7.5

(b)



Scorecard - Food Action Rating Test

Tray number ..... Name .....

You are presented with a food sample.
Please taste the sample and tick ✓ the box that best describes how you feel about it.

- ☐ I would eat this every opportunity that I had
- ☐ I would eat this very often
- ☐ 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 this but would eat it on occasion
- ☐ I would hardly ever eat this
- ☐ I would eat this only if forced to

©

Figure 4: Hedonic Rating Test and Questionnaire for First Phase (a, b) and second phase (c).

The results showed that the panelists thought that the texture could be improved, they thought that it was too brittle or crumbly. Some of the suggestions on the questionnaire were to use marshmallows to help the product bind better. A few persons also recommended that the almonds be replaced with sun flower seeds so that persons who are allergic to nuts can safely consume the product. For the Second Phase a Food Action/Attitude Rating test was done, see **Fig. 4.c** for sample of scorecard that was presented, a seven point scale (ranging from I would eat this every opportunity that I had to I would eat this only if forced to) was used to determine the attitudes of panelists to the snack. Assessors were asked to evaluate a sample of the modified granola bar and indicate which action best describes their feelings.

2.2.4 Types of Tests

Different sensory tests are employed for food evaluation. The tests are grouped into four types.

(i) **Difference tests:** Paired comparison test, duo trio test.

(ii) **Rating tests:** These tests give more quantitative data than difference tests and can be used for the analysis of more than two samples at the same time. For example: Ranking Test, Hedonic Rating Test.

(iii) **Sensitivity tests:** Sensitivity tests are done to assess the ability of individual to detect different tastes, odors and feel the presence of specific factors like astringency or hotness (pepper). These tests are used to select and train panel members for evaluating the quality of products containing spices, salt and sugar, e.g. tomato ketchup or sauce.

(iv) **Descriptive tests:** This is both qualitative and quantitative description method for flavor analysis in products containing different tastes and odor.

It is always very important to select the correct test method in sensory evaluation. The selection of a test method depends on:

1. **Test aims** - Sensory evaluation provides the following types of information that pertains to the different test aims:
    - Discriminative information - determination of differences between products/samples.
    - Descriptive information - focus on the description of products/samples. This information can be correlated with other forms of analysis such as chemical results etc.
    - Consumer information - focus on consumer preference, acceptance, liking etc. of one product over another. Ranking of one or more products or rating of products in a range (by consumers), can also be the aim.
2. **The sequence of events** - It is important to follow the correct procedure to identify the required method or test. A sequence of events can be used as a guideline during any phase of the product development process to prevent unnecessary and time-consuming testing, for example:
    - If there is a discernible difference - determine whether the difference is significant through discriminative tests such as the paired comparison, triangle, duo trio or ranking tests.
    - Determine which product is preferred and/or liked by consumers - representative consumers evaluate the samples using paired preference tests, hedonic scaling or ranking.
    - Obtain a summary of the characteristics of the sample or specific attributes and the relevant intensities - this will require trained panelists for Descriptive Analysis techniques such as Quantitative Descriptive Analysis, or Flavor profiling. Panelists with limited training can be used for Free Choice Profiling.
3. **The use of a decision tree** - for example the branched model proposed by Lawless and Heymann, 1998 in Singh Di & Maharaj., (2014), where the answer to a pertinent question leads to the identification of the most suitable test.

Table 2 provides a summary on which type of sensory evaluation method should be used based on the questions that you are trying to answer.

Table.2: Sensory Evaluation Questions and Methods

Questions	Sensory Evaluation Method	Basic Setup
Are products different?	Discrimination/	20-50 panelists
Which sample has greater intensity of an attribute? E.g. which is sweeter?	Difference Tests	Screened for acuity (keenness or sharpness of perception, i.e. can they smell and taste well?)
		Analysis is done using statistical tables which compare results to chance – this analysis ensures that the difference was real and not because people chose the correct sample by luck/chance.
		one-tailed binomial test, two-tailed binomial test and Chi Square test
If products are different, how are they different?	Descriptive Analysis	8-12 panelists or 6 to 10 panelist
		Screened for acuity, Trained
What is the magnitude of these differences?		Asked to rate intensity for all sensory attributes
		Analysis is done using a t-test or ANOVA to determine if means are statistically different.
What is the acceptability of a product? Is the product liked? Is one product preferred over another?	Affective/ Preference Hedonic Tests	75-150 consumers per test
		Min of 20 for pilot testing
		Screened for product use (Do they buy the product? And how often?)
		Asked degree of liking (how much do they like it) and/or preference questions
		Friedman test, t-test, 2 tailed binomial, ANOVA

2.2.5 Limitations of sensory evaluation

1. The result may be highly variable.
2. People with colds or other health problems temporarily lose their maximum effectiveness.
3. Emotional burdens may influence an individual's ability.

2.2.6 Interpretation of sensory results in statistical quality control

Once data are collected, they are in the form of a recorded list or a computer file. Organization and analysis require that the data are entered or loaded into an analysis package. For the majority of examples in this book, Excel is used followed by Minitab and reference is made to Megastat for Excel.

On the other hand, given the large number of possible sources of unwanted variation in such data (sensory and consumer), scientists must use experimental design to combat them.

2.3 Choice and validity of method

2.3.1 Objective of the essay and Choice of analytical methods

Selection of a method depends largely on the objective of the measurement. For example, methods used for rapid online processing measurements may be less accurate than official methods used for nutritional labeling purposes.

Primary methods: Methods referred to as reference, definitive, official or primary are most applicable in a well-equipped and staffed analytical lab.

Secondary methods: The more rapid secondary or field methods may be more applicable on the manufacturing floor in a food processing facility. For example, refractive index may be used as a rapid, secondary method for sugar analysis, with results correlated to those of the primary method, high-performance liquid chromatography (HPLC).

❖ **General criteria governing the choice of analytical methods**

- Preference should be given to official analysis methods developed by international organizations dealing with specific food or food groups.
- Preference should be given to analytical methods whose reliability has been established based on criteria such as:
  - ✓ Specificity.
  - ✓ Accuracy.
  - ✓ Precision: Intra-laboratory repeatability of results, inter-laboratory reproducibility of results.
  - ✓ Limit of detection.
  - ✓ Sensitivity.
  - ✓ Practical utility and applicability under normal laboratory conditions.
  - ✓ Other criteria as needed.
- Method selection should be based on practical utility, with preference given to methods applicable for routine purposes.
- Analytical methods applicable across various product groups should be preferred over methods applicable only to individual products.

2.3.2 Official methods and other endorsed methods

a- Official method

An official method is an analytical method accepted and recommended by international organizations that have evaluated its performance characteristics (precision, accuracy, etc.) through collaborative studies involving multiple laboratories worldwide.

b- Reference method

A reference method is an official method recognized by international organizations as the one that provides the most accurate result, meaning the closest to the true value of the concentration of a component under analysis. The reference method usually gives the most precise results when compared to other methods for analyzing the component.

AOAC International, formerly known as the **Association of Official Analytical Chemists (AOAC)**, is an organization begun in 1884 to serve the analytical methods needs of government regulatory and research agencies. The goal of AOAC International is to provide methods that will be fit for their intended purpose (i.e., will perform with the necessary accuracy and precision under usual laboratory conditions). Methods validated and adopted by AOAC International and the data supporting the method validation are published in the Journal of AOAC International.

Other Endorsed Methods:

- ✓ The AACC International formerly known as the **American Association of Cereal Chemists (AACC)** publishes a set of approved laboratory methods, applicable mostly to cereal products (e.g., baking quality, gluten, physical dough tests, and staleness/texture).
- ✓ The **American Oil Chemists' Society (AOCS)** has a reference sample program for oilseeds, oilseed meals, marine oils, aflatoxin, cholesterol, trace metals, specialty oils suitable for determination of Trans fatty acids, and formulations for nutritional labeling.

Part III.

3. Compositional analysis of foods

The goal of an assay is to determine the quantity of material, fraction, or concentration in a sample. There are three kinds of assays:

- **Physical assay methods** (spectrophotometric methods in UV, visible, or IR; nuclear physics methods).
- **Chemical assay methods or** Titrimetry (volumetry)**:** involving a chemical reaction between the analyte and a titrating reagent. In volumetric analysis, the substance being titrated reacts with an appropriate reagent, added in the form of a standard solution, and the volume of the solution required to complete the reaction is measured.
- **Physico-chemical assay methods** (electroanalytical methods, gravimetric methods, etc.). In gravimetric analysis, the substance to be measured is converted into an insoluble precipitate, which is collected and weighed.

3.1 Moisture and total solids analysis

Moisture assays can be one of the most important analyses performed on a food product and yet one of the most difficult from which to obtain accurate and precise data.

3.1.1 Importance of Moisture Assay

One of the most fundamental and important analytical procedures that can be performed on a food product is an assay for the amount of moisture. The dry matter that remains after moisture removal is commonly referred to as total solids. This analytical value is of great economic importance to a food manufacturer because water is an inexpensive filler. The following listing gives some examples in which moisture content is important to the food processor:

1. Moisture is a quality factor in the preservation of some products and affects stability in: (a) Dehydrated vegetables and fruits (b) Dried milks (c) Powdered eggs (d) Dehydrated potatoes (e) Spices and herbs.
2. Moisture is used as a quality factor for Jams and jellies to prevent sugar crystallization, sugar syrups.
3. Reduced moisture is used for convenience in packaging or shipping of concentrated milks, concentrated fruit juices.
4. Moisture (or solids) content is often specified in compositional standards (i.e., Standards of Identity) (a) Cheddar cheese must be ≤ 39 % moisture. (b) Enriched flour must be ≤ 15 % moisture.

5. Computations of the nutritional value of foods require that you know the moisture content.

6. Moisture data are used to express results of other analytical determinations on a uniform basis [i.e., dry weight basis (dwb), rather than wet weight basis (wwb)].

3.1.2 Forms of Water in Foods

The ease of water removal from foods depends on how it exists in the food product. The three states of water in food products are:

1. Free water: This water retains its physical properties and thus acts as the dispersing agent for colloids and the solvent for salts.
2. Adsorbed water: This water is held tightly or is occluded in cell walls or protoplasm and is held tightly to proteins.
3. Water of hydration: This water is bound chemically, for example, lactose monohydrate; also some salts such as $Na_2SO_4 \cdot 10H_2O$.

Depending on the form of the water present in a food, the method used for determining moisture may measure more or less of the moisture present However, several official methods may exist for a particular product. For example, the AOAC International methods for cheese include: Method 926.08, vacuum oven; 948.12, forced draft oven; 977.11, microwave oven; 969.19, distillation. Usually, the first method listed by AOAC International is preferred over others in any section.

3.1.3 Oven drying methods

In oven drying methods, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample. The amount of moisture determined is highly dependent on the type of oven used, conditions within the oven, and the time and temperature of drying. AOAC International approves various oven methods for determining the amount of moisture in many food products. The methods are simple, and many ovens allow for simultaneous analysis of large numbers of samples. The time required may be from a few minutes to over 24 h.

Calculations:

Moisture and total solids contents of foods can be calculated as follows using oven drying procedures:

$$\%Moisture\ (wt/wt) = \frac{wt\ H2O\ in\ sample}{wt\ of\ wet\ sample} \times 100$$

$$\%Moisture\ (wt/wt) = \frac{(wt\ of\ wet\ sample - wt\ of\ dry\ sample)}{wt\ of\ wet\ sample} \times 100$$

$$\%Total\ solids\ (wt/wt) = w\frac{t\ of\ dry\ sample}{wt\ of\ wet\ sample} \times 100$$

These thermogravimetric methods are the reference methods for determining water or total solids in foods. The analysis requires the use of a ventilated oven or a vacuum oven, along with a desiccator containing a drying agent.Conditions of heating and pressure:

- At 100-105 °C (ventilated oven) or 70-75 °C (vacuum oven).
- Atmospheric pressure (ventilated oven) or reduced pressure (vacuum oven).

3.1.3.1 Forced Draft Oven

When using a forced draft oven, the sample is rapidly weighed into a predried moisture pan covered and placed in the oven for an arbitrarily selected time if no standardized method exists. Drying time periods for this method are 0.75–24 h **(Table 3)**, depending on the food sample and its pretreatment; some liquid samples are dried initially on a steam bath at 100 °C to minimize spattering. In these cases, drying times are shortened to 0.75–3 h. A forced draft oven is used with or without a steam table predrying treatment to determine the solids content of fluid milks (AOAC Method 990.19, 990.20). An alternative to selecting a time period for drying is to weigh and reweigh the dried sample and pan until two successive weighing taken 30 min apart agree within a specified limit, for example, 0.1–0.2 mg for a 5-g sample. The user of this second method must be aware of sample transformation, such as browning which suggests moisture loss of the wrong form. Samples high in carbohydrates should not be dried in a forced draft oven but rather in a vacuum oven at a temperature no higher than 70 °C.

Table.3: Forced draft oven temperature and times for selected foods

Product	Dry on Steam Bath	Oven Temperature (°C ± 2)	Time in Oven (h)
Buttermilk, liquid	X[a]	100	3
Cheese, natural type only		100	16.5 ± 0.5
Chocolate and cocoa		100	3
Cottage cheese		100	3
Cream, liquid and frozen	X	100	3
Egg albumin, liquid	X	130	0.75
Egg albumin, dried	X	100	0.75
Ice cream and frozen desserts	X	100	3.5
Milk	X	100	3
Whole, low fat, and skim		100	3
Condensed skim		100	3
Nuts: almonds, peanuts, walnuts		130	3

aX=samplesmustbepartiallydriedonsteambathbeforebeingplacedinoven.

3.1.3.2 Vacuum Oven

By drying under reduced pressure (25–100 mmHg), one is able to obtain a more complete removal of water and volatiles without decomposition within a 3–6-h drying time. Vacuum ovens need a dry air purge in addition to temperature and vacuum controls to operate within method definition **(Fig.5)**.

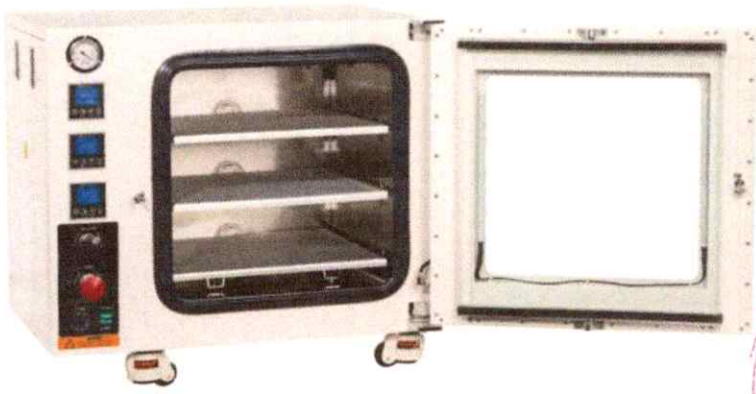


Figure. 5 Vacuum Oven Drying.

3.1.3.3 Microwave Analyzer

Microwave moisture analysis, often called microwave drying, was the first precise and rapid technique that allowed some segments of the food industry to make in-process adjustment of the moisture content in food products before final packaging. For example, processed cheese could be analyzed and the composition adjusted before the blend was dumped from the cooker. A particular microwave moisture/solids analyzer (CEM Corporation, Matthews, NC), or equivalent **(Fig.6)**, is specified in the AOAC International

procedures for total solids analysis of processed tomato products (AOAC Method 985.26) and moisture analysis of meat and poultry products (AOAC Method 985.14).

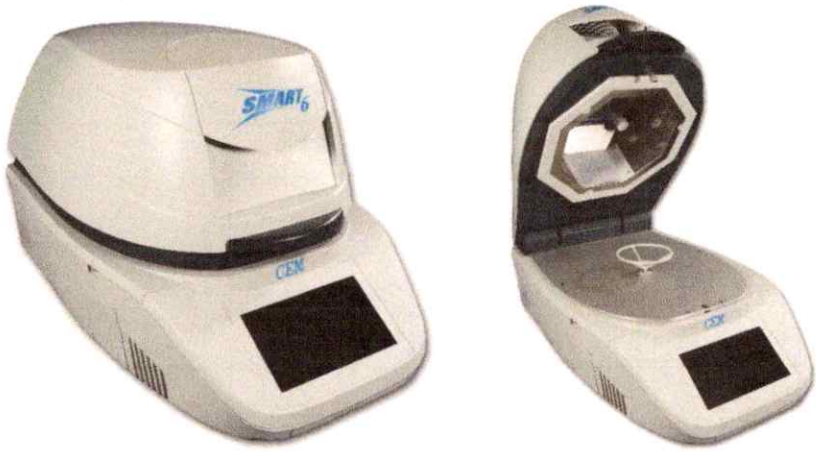


Figure.6 Microwave Infrared Moisture and Solids Analyzer.

There are some considerations when using a microwave analyzer for moisture determination:

(1) The sample must be of a uniform, appropriate size to provide for complete drying under the conditions specified;

(2) the sample must be centrally located and evenly distributed, so some portions are not burned and other areas are under processed; and.

(3) The amount of time used to place an appropriate sample weight between the pads must be minimized to prevent moisture loss or gain before weight determination.

3.1.3.4 Infrared Drying

Infrared drying **(Fig.7)** involves penetration of heat into the sample being dried, as compared with heat conductivity and convection with conventional ovens. Such heat penetration to evaporate moisture from the sample can significantly shorten the required drying time to 10–25 min. The infrared lamp used to supply heat to the sample results in a filament temperature of 2000–2500 K (degrees Kelvin). Factors that must be controlled include distance of the infrared source from the dried material and thickness of the sample. No infrared drying moisture analysis techniques are approved by AOAC International currently. However, because of the speed of analysis, this technique is suited for qualitative in-process use.

Figure. 7 Infrared drying apparatus.

3.1.4 Distillation procedures :

Distillation techniques involve co-distilling the moisture in a food sample with a high boiling point solvent that is immiscible in water, collecting the mixture that distills off, and then measuring the volume of water.

Two distillation procedures are in use today: direct and reflux distillations **(Fig.8)**, with a variety of solvents. For example, in direct distillation with immiscible solvents of higher boiling point than water, the sample is heated in mineral oil or liquid with a flash point well above the boiling point for water. Other immiscible liquids with boiling point only slightly above water can be used (e.g., toluene, xylene, and benzene). However, reflux distillation with the immiscible solvent toluene is the most widely used method.

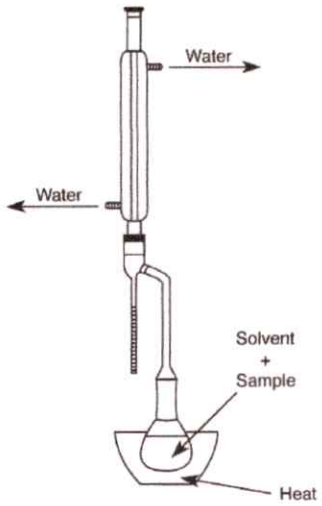


Figure.8 Apparatus for reflux distillation of moisture from a food.

This thermovolumetric method is used for determining moisture content in low-water-content foods (seeds, spices, etc.). The method directly measures the amount of water removed from the food. Water is removed by distillation with a immiscible solvent that forms an azeotropic mixture with water (water: boiling point T °C = 100 °C; C_6H_6, toluene, xylene: boiling point T °C = 140 °C; water + xylene: boiling point < 100 °C → the mixture boils at a lower temperature). The water removed from the sample is trapped in a graduated collector tube. When all the water is distilled, measure the volume of water collected in the graduated collector tube.

Three potential sources of error with distillation should be eliminated if observed:

1. Formation of emulsions that will not break. Usually this can be controlled by allowing the apparatus to cool after distillation is completed and before reading the amount of moisture in the trap.
2. Clinging of water droplets to dirty apparatus. Clean glassware is essential, but water seems to cling even with the best cleaning effort. A burette brush, with the handle end flattened so it will pass down the condenser, is needed to dislodge moisture droplets.
3. Decomposition of the sample with production of water. This is principally due to carbohydrate decomposition to generate water ($C_6H_{12}O_6 \rightarrow 6H_2O + 6C$). If this is a measurable problem, discontinue method use and find an alternative procedure.

3.1.5 Physical methods

3.1.5.1 Dielectric Method

The electrical properties of water are used in the dielectric method to determine the moisture content of certain foods, by measuring the change in capacitance or resistance to an electric current passed through a sample. These instruments require calibration against samples of known moisture content as determined by standard methods. These methods are limited to food systems that contain no more than 30–35 % moisture. The moisture determination in dielectric-type meters is based on the fact that the dielectric constant of water (80.37 at 20 °C) is higher than that of most solvents. The dielectric constant is measured as an index of capacitance. As an example, the dielectric method is used widely for cereal grains.

3.1.5.2 Hydrometry

Hydrometry is the science of measuring specific gravity or density, which can be done using several different principles and instruments. While hydrometryis considered archaic in some analytical circles, it is still widely used and, with proper technique, is highly accurate.

Specific gravity measurements with various types of hydrometers **(Fig.9)** or with a pycnometer are commonly used for routine testing of moisture (or solids) content of numerous food products. These include beverages, salt brines, and sugar solutions.

Figure. 9 Hydrometers. (Courtesy of Cole-Parmer Instrument Company, Vernon Hills, IL.)

The rudimentary but surprisingly accurate hydrometer comes equipped with various modifications depending on the fluid to be measured:

- Lactometer is used to determine the density of milk.
- Brix hydrometer is a type of saccharometer used for.
- Alcoholometers are used to estimate the alcohol content of beverages.
- The Baumé hydrometer was used originally to determine the density of salt solutions (originally 10 % salt), but it has come into much wider use.

3.1.5.3 Refractometry

Moisture in liquid sugar products and condensed milks can be determined using a Baumé hydrometer (solids), a Brix hydrometer (sugar content), gravimetric means, or a refractometer. If it is performed correctly and no crystalline solids are evident, the refractometer procedure is rapid and surprisingly accurate (AOAC Method 9.32.14C, for solids in syrups). The refractometer has been valuable in determining the soluble solids in fruits and fruit products (AOAC Method 932.12; 976.20; 983.17).

The refractive index (RI) of an oil, syrup, or other liquid is a dimensionless constant that can be used to describe the nature of the food. While some refractometers are designed only to provide results as refractive indices, others, particularly hand-held, quick-to-use units, are equipped with scales calibrated to read the percentage of solids, percentage of sugars, and the like, depending on the products for which they are intended.

All chemical compounds have an index of refraction. Therefore, this measurement can be used for the qualitative identification of an unknown compound by comparing its RI with literature values. RI varies with concentration of the compound, temperature, and wavelength of light. Instruments are designed to give a reading by passing a light beam of a specific wavelength through a glass prism into a liquid, the sample.

Bench-top or hand-held units **(Fig.10)** use Amici prisms to obtain the D line of the sodium spectrum or 589 nm from white light. Whenever refractive indices of standard fluids are given, these are prefaced with η20 D = a value from 1.3000 to 1.7000. The Greek letter η is the symbol for RI; the 20 refers to temperature in °C; and D is the wavelength of the light beam, the D line of the sodium spectrum.

Figure. 10 Rhino Brix hand-held refractometer. R2 mini digital hand-held refractometer, and Mark III Abbe refractometer. (Courtesy of Reichert Analytical Instrument, Depew, NY.).

3.1.6 Chemical assay: KARL FISCHER TITRATION

The Karl Fischer titration (German chemist) is particularly adaptable to food products that show erratic results when heated or submitted to a vacuum. This is the method of choice for determination of water in many low moisture foods (0.5 to 5 %), such as dried fruits and vegetables (AOAC Method 967.19 E-G), candies, chocolate (AOAC Method 977.10), roasted

coffee, oils and fats (AOAC Method 984.20), or any low-moisture food high in sugar or protein. The method is quite rapid, is accurate, and uses no heat.

This method is based on the fundamental reaction described by Bunsen in 1853 involving the reduction of iodine by SO_2 in the presence of water:

$$2H_2O + SO_2 + I_2 \rightarrow C_5H_2SO_4 + 2HI$$

This was modified to include methanol and pyridine in a four-component system to dissolve the iodine and SO_2:

$$C_5H_5N \cdot I_2 + C_5H_5N \cdot SO_2 + C_5H_5N + H_2O \rightarrow 2C_5H_5N \cdot HI + C_5H_5N \cdot SO_3$$

$$C_5H_5N \cdot SO_3 + CH_3OH \rightarrow C_5H_5N(H)SO_4 \cdot CH_3$$

This is a chemical method based on the oxidation of sulfurdioxide (SO_2) by iodine (I_2) in the presence of water. A small amount of the sample to be analyzed is placed in a reactive mixture containing sulfur dioxide and iodine.

These reactions show that for each mole of water, 1mol of iodine, 1 mol of SO_2, 3 mol of pyridine, and 1 mol of methanol are used. For general work, a methanolic solution is used that contains these components in the ratio of 1 iodine: 3 SO_2:10 pyridine, and at a concentration so that 3.5 mg of water = 1 ml of reagent.

The assay can be performed with two types of equipment: one coulometric, where the reagent is prepared by direct electrolysis in the titration cell, and the other volumetric, where the reagent is added using a burette. The difference between volumetry and coulometry is particularly due to the method of introducing iodine for titration.

In a volumetric titration procedure **(Fig.11),** iodine and SO_2 in the appropriate form are added to the sample in a closed chamber protected from atmospheric moisture. The excess of I_2 that cannot react with the water can be determined visually. The endpoint color is dark red-brown. The automated volumetric titration units (used for 100 ppm water to very high concentrations) use a pump for mechanical addition of titrant and use the conductometric method for endpoint determination (i.e., detection of excess iodine is by applying a current and measuring the potential). The moisture content of the sample is determined from the titration volume (mL). The end point is detected using the constant-current polarization voltage method.

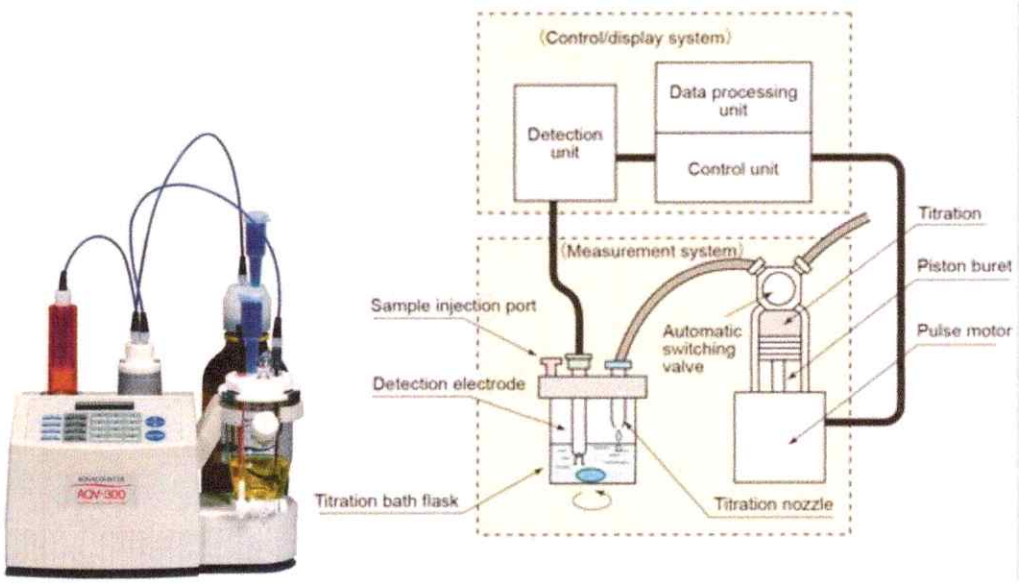


Figure 11: Automated Karl Fischer volumetric titration unit.

Today, pyridine has been replaced by imidazole because the obnoxious odor of pyridine makes it an undesirable reagent. The volumetric titration procedure described above is appropriate for samples with a moisture content greater than ~0.03 %.

A second type of titration, referred to as coulometric titration, is ideal for products with very low levels of moisture, from 0.03 % down to parts per million (ppm) levels. In this method, iodine is electrolytically generated ($2I \rightarrow I_2 + 2e-$) to titrate the moisture. The amount of iodine required to titrate the moisture is determined by the current needed to generate the iodine. According to Faraday's laws, the iodine is produced in proportion to the quantity of electricity. This means that the water content can be determined immediately from the coulombs required for electrolytic oxidation (1mg of water = 10.71 Coulombs).

Just like for volumetric titration, automated coulometric titration units are available commercially **(Fig.12)**.

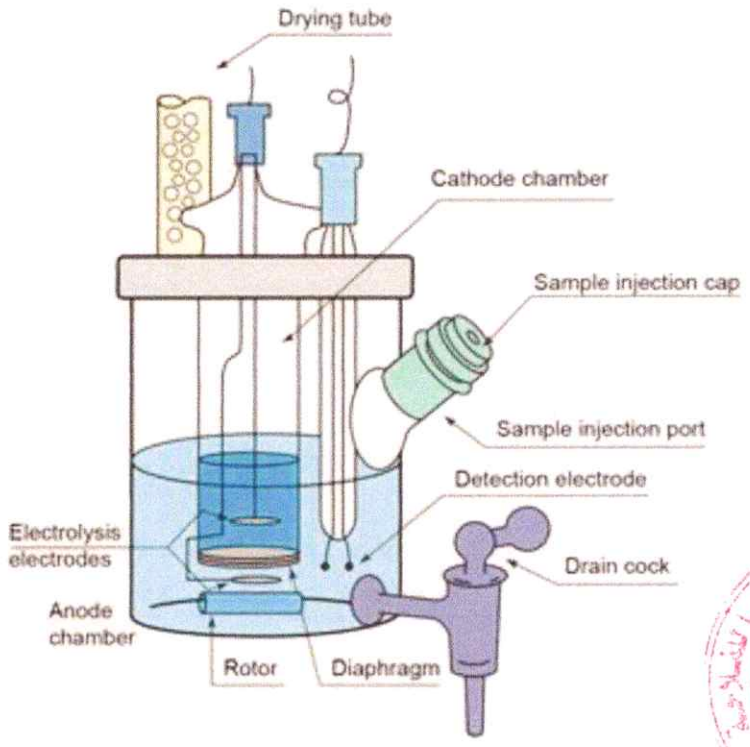


Figure 12: Electrolysis cell of Coulometric moisture meter.

3.2 Ash and Mineral Analysis

3.2.1 Importance of Ash in Food Analysis

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. The inorganic residue consists mainly of the minerals present in the food sample. Determining the ash content is part of the proximate analysis for nutritional evaluation. In addition, ashing is the first step in the preparation of a sample for specific elemental analysis. Two major types of ashing procedures are commonly used, dry ashing and wet ashing. Dry ashing is heating food at elevated temperatures (500–600 °C) in a muffle furnace (Food with high-moisture content, such as vegetables). In contrast, wet ashingis based on oxidizing organic matter using acids and oxidizing agents or their combination.

3.2.2 Ash Contents in Foods

The average ash content for various food groups is given in **Table (4)**. The ash content of most fresh foods rarely is greater than 5 % Carbohydrate Analysis.

Table.4: Ash content of selected foods

Food Item	*Percent Ash (Wet Weight Basis)*
Cereals, bread, and pasta	
Rice, brown, long-grain, raw	1.5
Corn meal, whole-grain, yellow	1.1
Hominy, canned, white	0.9
White rice, long-grain, regular, raw, enriched	0.6
Wheat flour, whole-grain	1.6
Macaroni, dry, enriched	0.9
Rye bread	2.5
Dairy products	
Milk, reduced fat, fluid, 2%	0.7
Evaporated milk, canned, with added vitamin A	1.6
Butter, with salt	2.1
Cream, fluid, half-and-half	0.7
Margarine, hard, regular, soybean	2.0
Yogurt, plain, low fat	1.1
Fruits and vegetables	
Apples, raw, with skin	0.2
Bananas, raw	0.8
Cherries, sweet, raw	0.5
Raisins	1.9
Potatoes, raw, skin	1.6
Tomatoes, red, ripe, raw	0.5
Meat, poultry, and fish	
Eggs, whole, raw, fresh	0.9
Fish fillet, battered or breaded, and fried	2.5
Pork, fresh, leg (ham), whole, raw	0.9
Hamburger, regular, single patty, plain	1.9
Chicken, broilers or fryers, breast meat only, raw	1.0
Beef, chuck, arm pot roast, raw	1.1

3.2.3 Sample Preparation

A 2-10 g sample generally is used for ash determination. For that purpose, milling, grinding, and the like probably will not alter the ash content much; however, if this ash is a preparatory step for specific mineral analyses, contamination by microelements is of potential concern. Remember, most grinders and mincers are of steel construction. The water source used in dilutions also may contain contaminants of some microelements. Distilled-deionized water always should be used. Plant materials are generally dried by routine methods prior to grinding. Animal products, syrups, and spices require treatments prior to ashing because of high fat, moisture (spattering, swelling), or high sugar content (foaming) that may result in loss of sample. Meats, sugars, and syrups need to be evaporated to dryness on a steam bath or with an infrared (IR) lamp.

3.2.4 Dry Ashing

Dry ashing is incineration at high temperature (525 °C or higher). Incineration is accomplished with a muffle furnace. Several models of muffle furnaces are available. Crucible selection becomes critical in ashing because the type depends upon the specific. Quartz crucibles are resistant to acids and halogens, but not alkali, at high temperatures. Pyrex R Gooch crucibles are limited to 500°C.

Procedures: AOAC International has several dry ashing procedures (e.g., AOAC Methods 900.02 A or B, 920.117, 923.03) for certain individual foodstuffs. The general procedure includes the following steps:

1. Weigh a 5–10-g sample into a tared crucible. Predry if the sample is very moist.
2. Place crucibles in a cool muffle furnace. Use tongs, gloves, and protective eyewear if the muffle furnace is warm.
3. Ignite 12–18 h (or overnight) at about 550 °C.
4. Turn off muffle furnace and wait to open it until the temperature has dropped to at least 250 °C, preferably lower. Open door carefully to avoid losing ash that may be fluffy.
5. Using safety tongs, quickly transfer crucibles to a desiccator with a porcelain plate and desiccant. Cover crucibles, close desiccator, and allow crucibles to cool prior to weighing.

The ash content is calculated as follows:

$$\%\ \boldsymbol{ash\ (dry\ basis)} = \frac{\boldsymbol{wt\ after\ ashing - tare\ wt\ of\ crucible}}{\boldsymbol{original\ sample\ wt \times dry\ matter\ coefficient}} \times \mathbf{100}$$

where: dry matter coefficient = % solids/100

For example, if corn meal is 87 % dry matter, the dry matter coefficient would be 0.87. If ash is calculated on an as received or wet weight basis (includes moisture), delete the dry matter coefficient from the denominator. If moisture was determined in the same crucible prior to ashing, the denominator becomes (dry sample wt - tared crucible wt).

3.2.5 Wet Ashing

Wet ashingis sometimes called wet oxidation or wet digestion. Its primary use is preparation for specific mineral analysis and metallic poisons. Often, analytical testing laboratories use only wet ashing in preparing samples for certain mineral analyses (e.g., Fe, Cu, Zn, P), because losses would occur by volatilization during dry ashing.

There are several advantages to using the wet ashing procedure: no loss from volatilization because of the lower temperature. The oxidation time is short and requires a hood, hot plate, and long tongs, plus safety equipment.

The disadvantages of wet ashing are that it takes virtually constant operator attention, corrosive reagents are necessary, and only small numbers of samples can be handled at any one time. Unfortunately, a single acid used in wet ashing does not give complete and rapid oxidation of organic material, so a mixture of acids often is used. Combinations of the following acid solutions are used most often: (1) nitric acid, (2) sulfuric acid-hydrogen peroxide, and (3) perchloric acid.

3.2.6 Soluble and insoluble ash (e.g., AOAC Method 900.02) – Applied to fruits Fat Analysis

Principle of the Method:

To determine the insoluble ash in water, the soluble part of the total ash is dissolved in hot water and filtered through a filter paper. The residue insoluble on the filter paper is incinerated again to burn the filter paper. The insoluble ash is then weighed. The percentage of soluble ash is calculated.

1) Calculations based on dry or wet sample

$$\%\ \boldsymbol{Insoluble\ Ash} = \frac{\boldsymbol{Wt(Insuluble\ Ash)\ x\ 100}}{\boldsymbol{Wt(Dry\ or\ Wet\ Sample)}}$$

$$\%\ \boldsymbol{Soluble\ Ash} = \%\ \boldsymbol{Total\ Ash} - \%\ \boldsymbol{Insoluble\ Ash}$$

2) Calculations based on total ash

$$\%\ \mathbf{Insoluble\ Ash} = \frac{\boldsymbol{Wt(Insuluble\ Ash)\ x\ 100}}{\boldsymbol{Wt(Total\ Ash)}}$$

$$\%\ \boldsymbol{Soluble\ Ash} = \frac{\boldsymbol{Wt(Soluble\ Ash)\ x\ 100}}{\boldsymbol{Wt(Total\ Ash)}}$$

3.2.7 Mineral Analysis

3.2.7.1 Importance of Minerals in the Diet

Calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulfur make up the dietary macro minerals, those minerals required at more than 100 mg/day by the adult. An additional ten minerals are required in milli- or microgram quantities per day and are referred to as trace minerals. These include iron, iodine, zinc, copper, chromium, manganese, molybdenum, fluoride, selenium, and silica. There is also a group of minerals called ultra trace minerals, including vanadium, tin, nickel, arsenic, and boron, that are being investigated

for possible biological function, but that currently do not have clearly defined biochemical roles. Some mineral elements have been documented to be toxic to the body and should, therefore, be avoided in the diet. These include lead, mercury, cadmium, and aluminum. Essential minerals such as fluoride and selenium also are known to be harmful if consumed in excessive quantities, even though they do have beneficial biochemical functions at proper dietary levels.

The Nutrition Labeling and Education Act of 1990 **(NLEA)** mandated labeling of sodium, iron, and calcium contents largely because of their important roles in controlling hypertension, preventing anemia, and impeding the development of osteoporosis, respectively.

The mineral content of water and foodstuffs is important because of their nutritional value, toxicological potential, and interactive effects with processing and texture of some foods. Traditional methods for mineral analysis include titrimetric and colorimetric procedures. These procedures generally require chemicals and equipment routinely available in an analytical laboratory and do not require expensive instrumentation. These methods may be suited to a small laboratory with skilled analytical personnel and a limited number of samples to be analyzed.

3.2.7.2 Methods

Separation of minerals from the food matrix is often specific, such as complexometric titrations or precipitation titrations. In these cases of specific separation, nonspecific measurements such as volume of titrant are made and are later converted to mass of mineral based on fundamental stoichiometric relationships. In other cases, separation of mineral involves nonspecific procedures such as ashing or acid extraction. These nonspecific separations require that a specific measurement be made as provided by **colorimetry**, **ion-selective electrodes (ISE)**, **atomic absorption spectroscopy**, or **inductively coupled plasma-atomic emission spectroscopy**.

3.2.7.3 Precipitation Titration : Mohr Titration of Salt in Butter (AOAC Method 960.29)

Salt in foods may be estimated by titrating the chloride ion with silver. The orange endpoint in this reaction occurs only when all chloride ion is complexed, resulting in an excess of silver to form the colored silver chromate. The endpoint of this reaction is therefore at the first hint of an orange color. When preparing reagents for this assay, use boiled water to

avoid interferences from carbonates in the water. Precipitation titration methods are well suited for any foods that may be high in chlorides. Because of added salt in processed cheeses and meats, these products should certainly be considered for using this method to detect chloride; then salt content is estimated by calculation. Precipitation titrations are easily automated, thus ensuring that these traditional methods will see continued use in the analytical food laboratory. For example, the automatic titration system commonly used to rapidly measure the salt content of potato chips is simply doing a Mohr titration.

3.2.7.4 Colorimetric Methods

Chromogens are chemicals that, upon reaction with the compound of interest, form a colored product. Chromogens are available that selectively react with a wide variety of minerals. Each chromogen reacts with its corresponding mineral to produce a soluble colored product that can be quantified by absorption of light at a specified wavelength. Generally, concentration of mineral in a sample is determined from a standard curve developed during the analysis, although in some cases it is possible to directly calculate concentration based on molar absorptivity of the chromogen–mineral complex. Samples generally must be ashed or treated in some other manner to isolate and/or release the minerals from organic complexes that would otherwise inhibit their reactivity with the chromogen. The mineral of interest must be solubilized from a dry ash and subsequently handled in a manner that prevents its precipitation. The soluble mineral may need to be treated (e.g., reduced or oxidized) to ensure that all mineral is in a form that reacts with the chromogen.

Colorimetric Determination of Iron in Meat: The total iron content of foods can be quantified spectrophotometrically as shown in **Fig. 13**. In this method, the absorption of light at 562 nm is converted to iron concentration in the sample via a regression equation generated from a standard curve developed during the analysis using a standard solution. The addition of ascorbic acid in the second to last step is necessary to convert all ionic iron to the detectable ferrous form. Repeating the procedure with and without ascorbic acid allows determination of total and ferrous ionic iron, respectively. Ferric iron is calculated by difference.

Applications: Colorimetry is used for the detection and quantification of a wide variety of minerals in food, and it is often a viable alternative to atomic absorption spectroscopy and other mineral detection methods.

IRON DETERMINATION OF MEAT—COLORIMETRIC ASSAY

Preparation of Standards

Prepare solutions of 10, 8, 6, 4, 2 µg iron/ml from a stock solution of 10 µg iron/ml.
Make dilutions using 0.1 *N* HCl.

Analysis of Sample

Place ~5 g sample into crucible and accurately weigh.

⇓

Heat on hot plate until well charred and sample has stopped smoking.

⇓

Ash in furnace at ca 550°C until ash is white.

⇓

Dissolve ash in small amount 1 *N* HCl and dilute to 50 ml volume with 0.1 *N* HCl.

⇓

Transfer 0.500 ml of diluted sample and standards into 10 ml test tubes.

⇓

Add 1.250 ml ascorbic acid (0.02% in 0.2 *N* HCl, made fresh daily). Vortex and let set 10 min.

⇓

Add 2.000 ml 30% ammonium acetate. Vortex. (pH needs to be >3 for color development)

⇓

Measure absorbance at 562 nm. Determine iron concentration in sample digest (µg iron/ml) from standard curve.

Figure. 13 Procedure for determination of iron in meat by colorimetry

3.3 Carbohydrate analysis :

Ingested carbohydrates are almost exclusively of plant origin, with milk lactose being the major exception. Of the monosaccharides (sometimes called simple sugars), only D-glucose and D-fructose are found in other than minor amounts. Monosaccharides are the only carbohydrates that can be absorbed from the small intestine. Higher saccharides (oligo- and polysaccharides) must first be digested (i.e., hydrolyzed to monosaccharides) before absorption and utilization can occur. At least 90 % of the carbohydrate in nature is in the form of polysaccharides. As stated above, starch polymers are the only polysaccharides that humans can digest and use as a source of calories and carbon. All other polysaccharides are nondigestible. Nondigestible polysaccharides can be divided into soluble and insoluble classes. Along with lignin and other nondigestible, nonabsorbed substances, they make up dietary fiber.

3.3.1 Sample preparation

Sample preparation is related to the specific raw material, ingredient, or food product being analyzed and the specific carbohydrate being determined, because carbohydrates have such a wide range of solubilities. However, some generalities can be presented **(Fig. 14)**. For most foods, the first step is drying, which also can be used to determine moisture content. Then, the material is ground to a fine powder, and lipids are extracted using 19:1 vol/vol chloroform–methanolin a Soxhlet extractor.

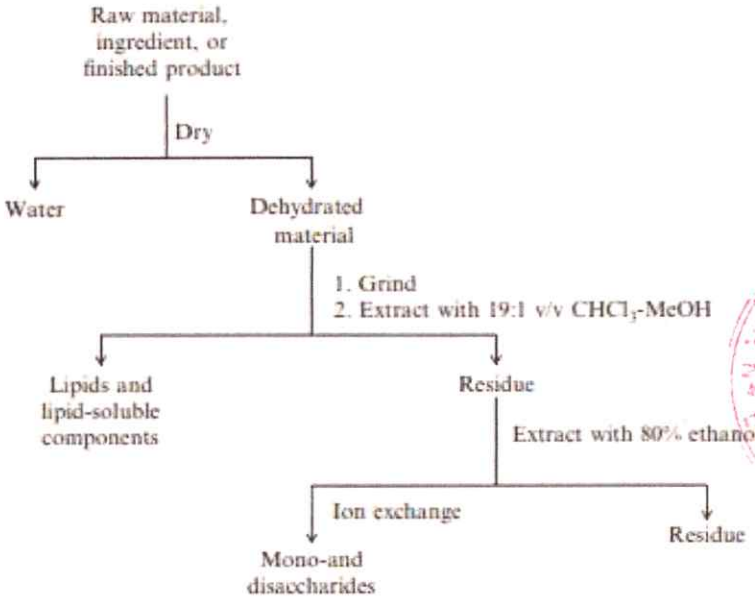
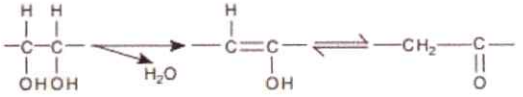


Figure 14: Flow diagram for sample preparation and extraction of mono- and disaccharides.

3.3.2 Total Carbohydrate: (Phenol-Sulfuric Acid Method 10.3.2.1)

3.3.2.1 Principle and Characteristics:

Strong acids and/or high temperatures destroy carbohydrates. Under these conditions, a series of complex reactions takes place, beginning with a simple dehydration reaction as shown in Equation [1].



[1]

Continued heating in the presence of acid produces various furan derivatives. These products then condense with themselves and other products to produce brown and black substances. They will also condense with various phenolic compounds, such as phenol, resorcinol, orcinol, α-naphthol, and napthoresorcinol, and with various aromatic amines, such as aniline and o-toluidine, to produce colored compounds that are useful for carbohydrate analysis.

The most often used condensation is with phenol itself (AOAC Method 44.1.30). This method is simple, rapid, sensitive, accurate, specific for carbohydrates, and widely applied. The reagents are inexpensive, readily available, and stable. Virtually all classes of sugars, including sugar derivatives and oligo- and polysaccharides, can be determined with the phenol-sulfuric acid method. A stable color is produced, and results are reproducible. Under proper conditions, the phenolsulfuric method is accurate to ± 2 %. The extent of reaction is, in

part, a function of the structure of the sugar. Therefore, a standard curve must be used. D-glucose is used to prepare the standard curve. In these cases, accuracy is determined by conformity of the standard curve made with D-glucose to the curve that would be produced from the exact mixture of carbohydrates being determined. If any concentrations are greater than the upper limit of the sensitivity range, dilutions should be used. The phenol-sulfuric acid procedure is often used as a qualitative test for the presence of carbohydrate.

3.3.2.2 Outline of Procedure

1. A clear, aqueous solution of carbohydrate(s) is transferred using a pipette into a small tube. A blank of water also is prepared.
2. An aqueous solution of phenol is added, and the contents are mixed.
3. Concentrated sulfuric acid is added rapidly to the tube so that the stream produces good mixing. The tube is agitated. (Adding the sulfuric acid to the water produces considerable heat.) A yellow-orange color results.
4. Absorbance is measured at 490 nm.
5. The average absorbance of the blanks is subtracted, and the amount of sugar is determined by reference to a standard curve.

3.3.3 Total Reducing Sugar

There are many methods for measuring carbohydrates. Some of these methods utilize the reducing or non-reducing power of sugars. The term "reducing sugars" historically comes from the chemical reaction that allowed their identification; the method was developed in the 19 th century by a German chemist, Hermann Fehling. Schematic representation shows that Fehling's solution initially contains copper(II) ions, which color the solution blue; certain sugars added to this solution can react with the copper(II) ions and convert them into copper(I) ions, which then produce a characteristic brick-red color. In chemistry, this transformation is called reduction, hence the term "reducing" for the sugars that trigger it.

The most common reducing sugars in foods are glucose and fructose, and to a lesser extent lactose and galactose. Table sugar or sucrose is not a reducing sugar, but if a sugar solution is heated and/or acidified, sucrose breaks down into glucose and fructose, which are reducing sugars. A mixture in equal quantities of D-glucose and D-fructose, obtained by the hydrolysis of sucrose.

$$\text{Sucrose} + H_2O \xrightarrow{\text{Hydrolysis}} \text{D-glucose} + \text{D-fructose}$$

In the food industry, the analysis of reducing sugars is very common and allows for the approximate quantification of simple sugars present. They are also analyzed because they are

reactive and can participate, particularly during thermal processes, in reactions known as Maillard reactions or caramelization.

3.3.4 Other methods

There are many methods which is based on total reducing sugar, like the Somogyi–Nelson method, are based on the reduction of Cu(II) ions in alkaline solution to Cu(I) ions that precipitate as the brick-red oxide Cu_2O. Tartrate or citrate ions are added to keep the Cu(II) ions in solution under the alkaline conditions. The Munson–Walker method (AOAC Method 906.03) has various forms. The precipitate of cuprous oxide can be determined gravimetrically (AOAC Method 31.039), by titration with sodium thiosulfate (AOAC Method 31.040), by titration with potassium permanganate (AOAC Method 31.042), by titration in the presence of methylene blue (the Lane–Eynon method; AOAC Method 923.09, 920.183b), and electrolytically (AOAC Method 31.044). These methods also must be used with standard curves because each reducing sugar reacts differently.

3.3.5 Enzymic methods for total starch

3.3.5.1 Principle

The only reliable method for determination of total starch is based on complete conversion of the starch into D-glucose by purified enzymes specific for starch and determination of the D-glucose released by an enzyme specific for it. Starch-hydrolyzing enzymes (amylases) must be purified to eliminate any other enzymic activity that would release D-glucose (e.g., cellulases, invertase or sucrase, β-glucanase) and catalase, which would destroy the hydrogen peroxide on which the enzymic determination of D-glucose depends.

3.3.5.2 Degree of gelatinization of starch

When starch granules are heated in water to a temperature specific for the starch being cooked, they swell, lose their crystallinity and birefringence, and become much more susceptible to enzyme-catalyzed hydrolysis. Heating starch in water produces phenomena that result from two processes: gelatinization and pasting, often together referred to simply as gelatinization, which are very important in determining the texture and digestibility of foods containing starch. Several methods have been developed that make use of the fact that certain enzymes act much more rapidly on cooked starch than they do on native starch. A particularly sensitive method employs a combination of pullulanase and β-amylase, neither of which is able to act on uncooked starch granules. With gelatinized or pasted starch, the enzyme pullulanasedebranches amylopectin and any branched amylose molecules, giving a mixture of

linear segments of various sizes. (Another debranching enzyme, isoamylase, may also be used.) β-Amylase then acts on the linear chains, releasing the disaccharide maltose, starting at the nonreducing ends **(Fig.15)** and a small amount of maltotriose (from chains containing an odd number of glucosyl units). The degree of gelatinization is determined by measuring the amount of reducing sugar formed.

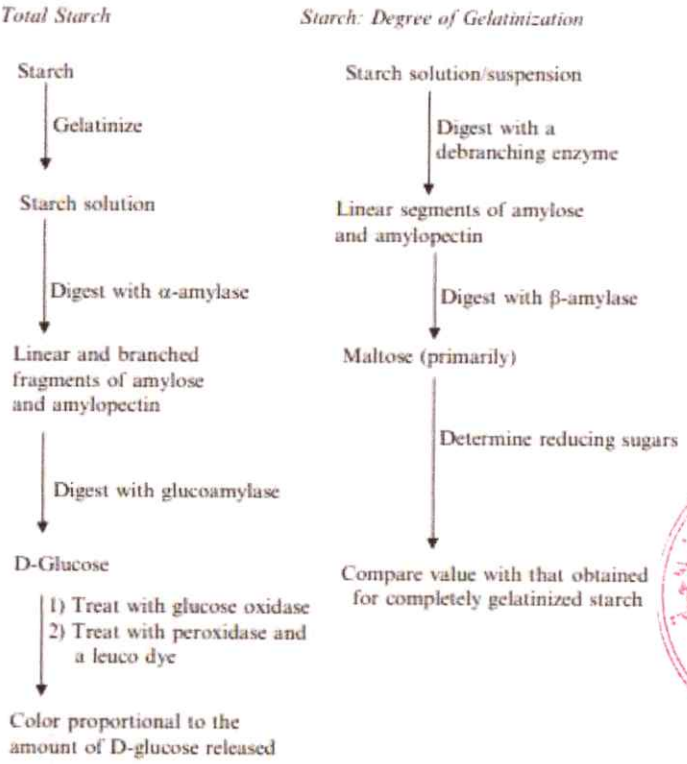


Figure 15: Flow diagrams for determination of total starch and determination of the degree of starch gelatinization.

3.3.5.3 Degree of retrogradation of starch

Upon storage of a product containing cooked starch, the two starch polymers, amylose and amylopectin, associate with themselves and with each other, forming polycrystalline arrays. This process of reordering is calledretrogradation. (Retrogradation is a contributing factor to the staling of bread and other bakery products, for example.) Retrograded starch, like native starch, is acted on very slowly by the combination of pullulanase plus β-amylase. The decrease in reducing power (from maltose released by action of the enzyme combination) after storage is a measure of the amount of retrograded starch at the time of analysis and/or the degree of retrogradation.

3.4 Fat Analysis

The lipid content in bovine milk **(Table 5)** illustrates the complexity and variability of lipids in a food system, having lipids that differ in polarity and concentrations. Foods contain many types of lipids, but those which tend to be of greatest importance are the triacylglycerols and the phospholipids. Liquid triacylglycerols at room temperature are referred to as oils, such as soybean oil and olive oil, and are generally of plant origin. Solid triacylglycerols at room temperature are termed as fats. Lard and tallow are examples of fats, which are generally from animals. The term fat is applicable to all triacylglycerols whether they are normally solid or liquid at ambient temperatures.

Table 5: Lipids of Bovine Milk

Kinds of Lipids	*Percent of Total Lipids*
Triacylglycerols	97–99
Diacylglycerols	0.28–0.59
Monoacylglycerols	0.016–0.038
Phospholipids	0.2–1.0
Sterols	0.25–0.40
Squalene	Trace
Free fatty acids	0.10–0.44
Waxes	Trace
Vitamin A	(7–8.5 µg/g)
Carotenoids	(8–10 µg/g)
Vitamin D	Trace
Vitamin E	(2–5 µg/g)
Vitamin K	Trace

An accurate and precise quantitative and qualitative analysis of lipids in foods is important for accurate nutritional labeling, determination of whether the food meets the standard of identity, and to ensure that the product meets manufacturing specifications. The validity of the fat analysis of a food depends on proper sampling and preservation of the sample before the analysis.

The extraction efficiency of lipids from dried foods depends on particle size; therefore, adequate grinding is very important. The classical method of determining fat in oilseeds involves the extraction of the ground seeds with selected solvent after repeated grinding at low temperature to minimize lipid oxidation. For better extraction, the sample and solvent are mixed in a high-speed comminuting device such as a blender.

3.4.1 Semicontinuous solvent extraction method: Soxhlet Method

The Soxhlet method (AOAC Method 920.39C for Cereal Fat; AOAC Method 960.39 for Meat Fat) (8) is an example of the semicontinuous extraction method and is described below.

3.4.1.1 Preparation of Sample

If the sample contains more than 10 % H_2O, dry the sample to constant weight at 95–100 °C under pressure ≤ 100 mm Hg for about 5 h (AOAC Method 934.01).

3.4.1.2 Procedure (See Fig.16)

1. Weigh, to the nearest mg, about 2 g of predried sample into a predried extraction thimble, with porosity permitting a rapid flow of ethyl ether. Cover sample in thimble with glass wool.
2. Weigh predried boiling flask.
3. Put anhydrous ether in boiling flask. Note: The anhydrous ether is prepared by washing commercial ethyl ether with two or three portions of H_2O, adding NaOH or KOH, and letting stand until most of H_2O is absorbed from the ether. Add small pieces of metallic Na and let hydrogen evolution cease (AOAC Method 920.39B). Petroleum ether may be used instead of anhydrous ether (AOAC Method 960.39).
4. Assemble boiling flask, Soxhlet flask, and condenser.
5. Extract in a Soxhlet extractor at a rate of five or six drops per second by condensation for about 4 h, or for 16 h at a rate of two or three drops per second by heating solvent in boiling flask. The separation of the solvent from the extract is done using the device called rotary evaporator (Rotavapor) **(Fig.16)**. The solution to be concentrated is placed in the round-bottom Flask A in a water bath (E) at a controlled temperature. The system is evacuated by means of a water aspirator or pump; connecting tubing is attached at the arrow. Flask (A) turns (generally slowly). Evaporation is relatively rapid from a thin film on the inside walls of flask (A) produced by its rotation because of the reduced pressure, the large surface area, and the elevated temperature. C is a condenser. D is the motor. Condensate collects in flask B. The stopcock at the top of the condenser is for releasing the vacuum.
6. Dry boiling flask with extracted fat in an air oven at 100 °C for 30 min, cool in desiccator, and weigh.

Calculation:

$$\%\ \boldsymbol{Fat\ on\ dry\ weight\ basis} = (\boldsymbol{g\ of\ fat\ in\ sample / g\ of\ dried\ sample}) \times \mathbf{100}$$

Influencing factors on the precision and accuracy of results:

1. Extraction time of the fat material. Too short an extraction time gives inaccurate results, i.e., lower than expected results.
2. Size of particles of solid food. The food should be ground to offer the largest possible contact surface to the extracting solvent.
3. Incomplete evaporation of the solvent before weighing the fat material.
4. Quality of the extracting solvent.

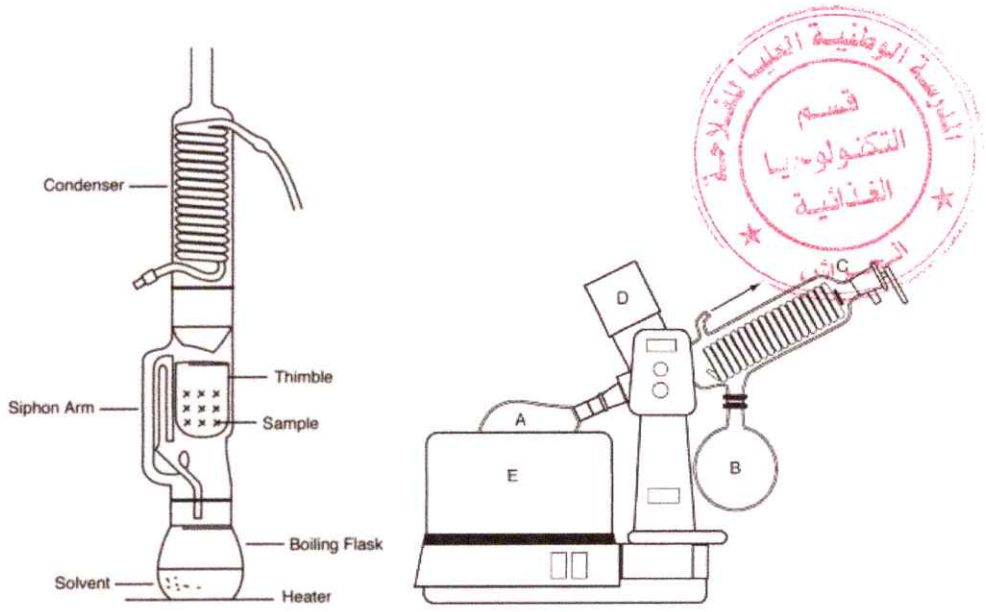


Figure 16: Soxhlet extraction apparatus (Left).Diagram of a rotary evaporator. (Right).

3.4.2 Discontinuous solvent extraction methods (Mojonnier Method)

3.4.2.1 Principle and Characteristics

Fat is extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat is dried to a constant weight and expressed as percent fat by weight. The Mojonnier test is an example of the discontinuous solvent extraction method and does not require removal of moisture from the sample. It can be applied to both liquid and solid samples.

The Mojonnier method was developed for and is applied primarily to dairy foods (procedure as described below for milk fat), but is applicable to other foods. Specifically, methods for fat in flour (AOAC Method 922.06) and fat in pet food (AOAC Method 954.02) both involve an acid hydrolysis with HCl, followed by extraction with a combination of ethyl ether and petroleum ether as described in AOAC Method 989.05 below for milk fat.

The Mojonnier method is the reference method for determining fat in dairy products. This gravimetric method, an adaptation of the Roëse-Gotlieb method, uses a special device, the Mojonnier apparatus.

The dairy product is weighed and then dissolved in the aqueous phase containing ammonium hydroxide and ethyl alcohol. Fat is extracted using an organic solvent immiscible with water, composed of ethyl ether and petroleum ether. The organic phase is decanted into a dish, the solvent evaporated, and the fat weighed.

3.4.2.2 Procedure: Milk Fat Method (AOAC Method 989.05)

1. **Preparation of Sample:** Bring the sample to about 20 °C; mix to prepare a homogeneous sample by pouring back and forth between clean beakers. Promptly weigh or measure the test portion. If lumps of cream do not disperse, warm the sample in a water bath to about 38 °C and keep mixing until it is homogeneous, using a "rubber policeman" if necessary to reincorporate the cream adhering to the container or stopper. When it can be done without interfering with dispersal of the fat, cool warmed samples to about 20 °C before transferring the test portion.
2. **Procedure**

(a) Weigh, to the nearest 0.1 mg, 10 g of milk into a Mojonnier fat extraction flask (**Fig. 17**). (b) Add 1.5 ml of NH_4OH and shake vigorously. Add 2 ml if the sample is sour. NH4OH neutralizes the acidic sample and dissolves protein.

(c) Add 10 ml of 95 % ethanol and shake for 90 s. The alcohol prevents possible gel formation.

(d) Add 25 ml of ethyl ether and shake for 90 s. The ether dissolves the lipid.

(e) Cool if necessary, and add 25 ml of petroleum ether and shake for 90 s. The petroleum ether removes moisture from the ethyl ether extract and dissolves more nonpolar lipid.

(f) Centrifuge for 30 s at 600 rpm.

(g) Decant ether solution from the Mojonnier flask into the previously weighed Mojonnier fat dish.

(h) Perform second and third extractions in the same manner as for the first extraction described previously (ethanol, ethyl ether, petroleum ether, centrifugation, decant).

(i) Evaporate the solvent in the dish on the electric hot plate at ≤100 °C in a hood.

(j) Dry the dish and fat to a constant weight in a forced air oven at 100 °C ± 1 °C.

(k) Cool the dish to room temperature and weigh.

Figure 17: Mojonnier fat extraction flask. (Courtesy of Kontes Glass Co., Vineland, NJ.).

3. Calculations

$$\%\,\boldsymbol{Fat} = \mathbf{100} \times \{[(\boldsymbol{wt\ dish} + \boldsymbol{fat}) - (\boldsymbol{wt\ dish})] - (\boldsymbol{avgwt\ blank\ residue})\}/\boldsymbol{wt\ sample}$$

$$\%\,\boldsymbol{Fat} = (\boldsymbol{Wt\ Fat}\ /\ \boldsymbol{Wt\ sample})\ \boldsymbol{x}\ \mathbf{100}$$

4. Specificity of the method:

Due to the variability fat content in dairy products, the amount of sample weighed, the number of extractions, and the volume of solvent used must be adjusted according to each type of dairy product.

- Method Precision:
  - ± 0.02 % for skim milk;
  - ± 0.03 % for other milks;
  - ± 0.1% for creams.
- Role of reagents:
  - Ammonium hydroxide (NH_4OH) with relative density 0.8974:

    Neutralizes the acidity of the dairy product, reduces viscosity, thus facilitating the action of solvents, and prevents gel formation.
  - 95% ethyl alcohol:

    Facilitates extraction, as alcohol is miscible with ether in any proportion; breaks any bond between proteins and phospholipids, which are then included with fat; facilitates separation of the aqueous phase and organic phase.
  - Ethyl ether (35°C):

    Dissolves fat and keeps it in ether solution. Ether also dissolves some water containing a small amount of non-fat solids that can cause erroneous results unless subsequent correction is made.
  - Petroleum ether 40-60 °C:

Petroleum ether is a mixture of hydrocarbons with boiling points between 40 and 60 °C and is used to remove any water traces from the ether solution that may contain non-fat solids.

- Influencing factors on the precision and accuracy of results:

1. Dairy products containing thickening agents:

Some dairy products contain thickening agents, which cause emulsions between the aqueous phase and the organic phase. The results obtained are non-reproducible.

2. Position of the interface between the two phases before decantation.

3. Quality of organic solvents:

When purchasing any new container of ethyl ether or petroleum ether, solvent quality is checked by performing a blank. The total volume of ethyl ether and petroleum ether normally used for analysis should not contain more than 0.5 mg of non-evaporable residue.

4. Accidental decantation of the aqueous phase:

The aqueous phase contains all hydrosoluble milk solids (proteins, lactose, minerals, etc.). Any accidental decantation of the aqueous phase increases the fat mass and gives a higher result than the expected result.

3.4.3 Nonsolvent wet extraction methods Babcock Method for Milk Fat (AOAC Method 989.04 and 989.10)

3.4.3.1 Principle:

In the Babcock method, H_2SO_4 is added to a known amount of milk in the Babcock bottle. The sulfuric acid digests protein, generates heat, and releases the fat. Centrifugation and hot water addition isolate fat for quantification in the graduated portion of the test bottle. The fat is measured volumetrically, but the result is expressed as percent fat by weight.

3.4.3.2 Procedure

1. Accurately pipette the milk sample (17.6 ml) into a Babcock test bottle **(Fig.18)**.
2. Add reagent grade (1.82, specific gravity) sulfuric acid (17.5 ml) to the bottle, allowing the acid to flow gently down the neck of the bottle as it is being slowly rotated. The acid digests proteins to liberate the fat.
3. Centrifuge the mixture for 5 min and liquid fat will rise into the calibrated bottle neck. The centrifuge must be kept at 55–60 °C during centrifugation.

4. Add hot water to bring liquid fat up into the graduated neck of the Babcock bottle.
5. The direct percentage of fat by weight is read to the nearest 0.05 % from the graduation mark of the bottle.

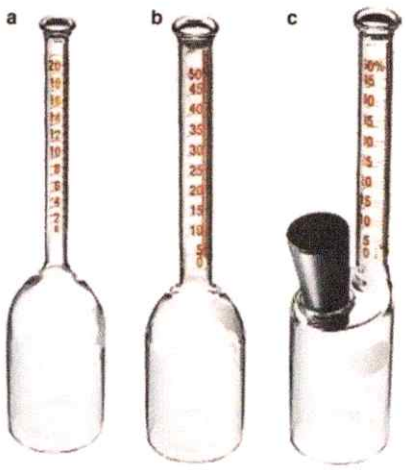


Figure 18: Babcock milk test bottles for milk (a), cream (b), and cheese (Paley bottle) (c) testing. (Courtesy of Kimble Glass Co., Vineland, NJ.

3.4.4 Gerber Method for Milk Fat

3.4.4.1 Principle

The principle of the Gerber method is similar to that of the Babcock method, but it uses sulfuric acid and amyl alcohol. The sulfuric acid digests proteins and carbohydrates, releases fat, and maintains the fat in a liquid state by generating heat.

3.4.4.2 Procedure

1. Transfer 10 ml of H_2SO_4 at 15–21 °C into a Gerber milk bottle.
2. Accurately measure milk sample (11 ml) into the Gerber bottle, using a Gerber pipette.
3. Add 1 ml of isoamyl alcohol to the bottle.
4. Tighten the stopper and mix by shaking the bottle.
5. Centrifuge the bottle for 4 min.
6. Place the bottle in a water bath at 60–63 °C for 5 min and then read the fat content from the graduations on the bottle neck.

3.4.4.3 Applications

The Gerber method is comparable to the Babcock method but is simpler and faster and has wider application to a variety of dairy products. The isoamyl alcohol generally prevents the charring of sugar found with the regular Babcock method. This test is more popular in Europe than in America.

3.5 Protein Analysis

3.5.1 Importance of Analysis

Protein analysis is important for:

1. Nutrition labeling.
2. Pricing: The cost of certain commodities is based on the protein content as measured by nitrogen content (e.g., cereal grains; milk for making certain dairy products, e.g., cheese).
3. Functional property investigation: Proteins in various types of food have unique food functional properties: for example, gliadin and glutenins in wheat flour for breadmaking, casein in milk for coagulation into cheese products.
4. Biological activity determination: Some proteins, including enzymes or enzyme inhibitors, are relevant to food science and nutrition.

3.5.2 Kjeldahl Method:

Unlike sugars and lipids, proteins contain nitrogen. This property is utilized in the method for determining protein content in foods.

The Kjeldahl method is the reference method for determining protein in foods. There are two versions of the method that use the same principle: the macro-Kjeldahl method and the micro-Kjeldahl method. They differ only in the apparatus used and the quantities of sample; the mass of sample analyzed by the macro-Kjeldahl method is about 5 times higher than that analyzed by the micro-Kjeldahl method.

General Procedures and Reactions:

1. **Sample Preparation:** Solid foods are ground to pass a 20-mesh screen. Samples for analysis should be homogeneous. No other special preparations are required.
2. **Digestion:** Place sample (accurately weighed) in a Kjeldahl flask. Add acid and catalyst; digest until clear to get complete breakdown of all organic matter. Nonvolatile ammonium sulfate is formed from the reaction of nitrogen and sulfuric acid. Adding catalyst K_2SO_4 (potassium sulfate) aims to raise the boiling point of the solution to accelerate the mineralization reaction of organic matter.

$$\text{Protein} \xrightarrow[\text{Heat, catalyst}]{\text{Sulfuric acid}} (NH_4)_2SO_2 \qquad [1]$$

3. **Neutralization and Distillation:** The digest is diluted with water. Alkali-containing sodium thiosulfate is added to neutralize the sulfuric acid. The ammonia formed is

distilled into a boric acid solution containing the indicators methylene blue and methyl red (AOAC Method 991.20).

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \quad [2]$$

$$NH_3 + H_3BO_3 \text{ (boric acid)} \rightarrow NH_4 + H_2BO_3^- \text{ (borate ion)} \quad [3]$$

4. **Titration:** Borate anion (proportional to the amount of nitrogen) is titrated with standardized HCl.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3 \quad [4]$$

5. **Calculations:**

Moles of HCl = moles of NH3 = moles of N in the sample [5]

A reagent blank should be run to subtract reagent nitrogen from the sample nitrogen

$$\%\,N = N\,HCl \times \frac{\text{Corrected acid volume}}{\text{g of sample}} \times \frac{14\,g\,N}{mol} \times 100 \quad [6]$$

Where:

- N HCl = normality of HCl, in mol/1000 ml.
- Corrected acid vol. = (ml std. acid for sample) – (ml std. acid for blank).
- 14 = atomic weight of nitrogen.
- A factor is used to convert percent N to percent crude protein. Most proteins contain 16% N, so the conversion factor is 6.25 (100/16 = 6.25).

$$\%\,\boldsymbol{N/0.16} = \%\,\boldsymbol{protein} \quad [7]$$

$$\text{Or} \quad \%\,\boldsymbol{N} \times \boldsymbol{6.25} = \%\,\boldsymbol{protein}$$

Conversion factors for various foods are given in **Table 6**.

Table 6: Nitrogen to protein conversion factors for various foods.

	Percent N in Protein	*Factor*
Egg or meat	16.0	6.25
Milk	15.7	6.38
Wheat	18.76	5.33
Corn	17.70	5.65
Oat	18.66	5.36
Soybean	18.12	5.52
Rice	19.34	5.17

6.Applications

Advantages:

1. Applicable to all types of foods.
2. Inexpensive (if not using an automated system).
3. Accurate; an official method for crude protein content.
4. Has been modified (micro Kjeldahl method) to measure microgram quantities of proteins.

Disadvantages:

1. Measures total organic nitrogen, not just protein nitrogen.
2. Time consuming (at least 2 h to complete).
3. Poorer precision than the biuret method.
4. Corrosive reagent.

3.5.3 Biuret method:

3.5.3.1 Principle:

A violet-purplish color is produced when cupric ions are complexed with peptide bonds (substances containing at least two peptide bonds, i.e., biuret, large peptides, and all proteins) under alkaline conditions **(Fig.19)**. The absorbance of the color produced is read at 540 nm. The color intensity (absorbance) is proportional to the protein content of the sample.

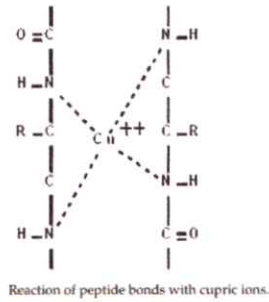


Figure. 19 Reaction of peptide bonds with cupric ions

This method was developed by Gornall et al. (1949) who applied the biuret reaction to obtain a quantitative method for protein assay.

3.5.3.2 Procedure:

1. A 5-ml biuret reagent is mixed with a 1-ml portion of protein solution (1–10 mg protein/ml). The reagent includes copper sulfate, NaOH, and potassium sodium tartrate, which is used to stabilize the cupric ion in the alkaline solution.
2. After the reaction mix is allowed to stand at room temperature for 15 or 30 min, the absorbance is read at 540 nm against a reagent blank.
3. Filtration or centrifugation before reading absorbance is required if the reaction mixture is not clear.
4. A standard curve of concentration versus absorbance is constructed using bovine serum albumin (BSA).

The staining reagent used is the Gornall reagent, composed of:

- Copper sulfate, which gives the blue color of the reagent due to copper ions at 3mmol/L;
- A 0.2 mol/L sodium hydroxide solution (soda) which makes the medium basic;
- Sodium and potassium double tartrate, which chelates (traps) Cu^{2+} ions and prevents their precipitation in alkaline medium as insoluble copper hydroxide $Cu(OH)_2$;
- Potassium iodide, to prevent the reduction of cupric ions before titration.

Although the reading is spectrophotometric, it is necessary to carry out a calibration range:

The protocol for performing an assay by the biuret method is as follows: mix 3 ml of the Gornall reagent with 2 ml of various protein solutions of known concentrations. Then incubate at 37 °C for 30 min in the dark. Allow to cool and then measure the absorbances of the solutions at 540 nm. This allows obtaining a standard range and plotting the standard curve to determine the protein concentration of an unknown solution treated in the same way.

3.5.3.3 Advantages:

1. Less expensive than the Kjeldahl method; rapid (can be completed in less than 30 min); simplest method for analysis of proteins.
2. Color deviations are encountered less frequently than with Lowry, ultraviolet (UV) absorption, or turbidimetric methods (described below).
3. Very few substances other than proteins in foods interfere with the biuret reaction.
4. Does not detect nitrogen from nonpeptide or nonprotein sources.

3.5.3.4 Disadvantages:

1. Not very sensitive as compared to the Lowry method; requires at least 2–4 mg protein for assay.
2. Absorbance could be contributed from bile pigments if present.
3. High concentration of ammonium salts interfere with the reaction.
4. Color varies with different proteins; gelatin gives a pinkish-purple color.
5. Opalescence could occur in the final solution if high levels of lipid or carbohydrate are present.
6. Not an absolute method: color must be standardized against known protein (e.g., BSA) or against the Kjeldahl nitrogen method.

3.5.4 Lowry method

Principle: The Lowry method combines the biuret reaction with the reduction of the Folin–Ciocalteau phenol reagent (phosphomolybdic-phosphotungstic acid) by tyrosine and tryptophan residues in the proteins. The bluish color developed is read at 750 nm (high sensitivity for low protein concentration) or 500 nm (low sensitivity for high protein concentration).

Because of its simplicity and sensitivity, the Lowry method has been widely used in protein biochemistry. However, it has not been widely used to determine proteins in food systems without first extracting the proteins from the food mixture.

3.5.5 Bradford Dye-Binding Method

3.5.5.1 Principle:

When Coomassie Brilliant Blue G-250 binds to protein, the dye changes color from reddish to bluish and the absorption maximum of the dye is shifted from 465 to 595 nm. The change in the absorbance at 595 nm is proportional to the protein concentration of the sample. Like other dye-binding methods, the Bradford relies on the amphoteric nature of proteins.

3.5.5.2 Procedure

1. Coomassie Brilliant Blue G-250 is dissolved in 95 % ethanol and acidified with 85 % phosphoric acid.
2. Samples containing proteins (1–100 µg/ml) and standard BSA solutions are mixed with the Bradford reagent.
3. Absorbance at 595 nm is read against a reagent blank.
4. Protein concentration in the sample is estimated from the BSA standard curve.

3.5.5.3 Applications:

The Bradford method has been used successfully to determine protein content in worts and beer products and in potato tubers. This procedure has been improved to measure microgram quantities of proteins. Due to its rapidity, sensitivity, and fewer interferences than the Lowry method, the Bradford method has been used widely for the analysis of low concentrations of proteins and enzymes in their purification and characterizations.

3.6 Enzyme activity assays

Enzymes are protein catalysts that are capable of very great specificity and reactivity under physiological conditions. Enzymatic analysis is the measurement of compounds with the aid of added enzymes or the measurement of endogenous enzyme activity to give an indication of the state of a biological system including foods. There are several uses of enzyme analyses in food science and technology. In several instances, enzyme activity is a useful measure for adequate processing of a food product.

The thermal stability of enzymes has been used extensively as a measure of heat treatment; for example, peroxidase activity is used as a measure of adequacy of blanching of vegetable products.

The food scientist can also use commercially available enzyme preparations to measure constituents of foods that are enzyme substrates. For example, glucose content can be determined in a complex food matrix containing other monosaccharides by using readily available enzymes.

3.6.1 Enzyme kinetics

Enzymes are biological catalysts that are proteins. A catalyst increases the rate (velocity) of a thermodynamically possible reaction. The enzyme does not modify the equilibrium constant of the reaction, and the enzyme catalyst is not consumed in the reaction. Because enzymes affect rates (velocities) of reactions, some knowledge of enzyme kinetics (study of rates) is needed for the food scientist to effectively use enzymes in analysis. To measure the rate of an enzyme-catalyzed reaction, typically one mixes the enzyme with the substrate under specified conditions (pH, temperature, ionic strength, etc.) and follows the reaction by measuring the amount of product that appears or by measuring the disappearance of substrate. Consider the following as a simple representation of an enzyme-catalyzed reaction:

$$S + EES \rightarrow P + E$$

Where:

- S= substrate.
- E= enzyme.
- ES =enzyme–substratecomplex P = product.

The rate of an enzyme-catalyzed reaction depends on the concentration of the enzyme and also depends on the substrate concentration. The velocity of the reaction at this very large substrate concentration is the maximum velocity (Vm) of the reaction under the conditions of that particular assay. The substrate concentration at which one-half Vmis observed is defined as the Michaelis constant or Km. Km is an important characteristic of an enzyme. It is an indication of the relative binding affinity of the enzyme for a particular substrate. The lower the Km, the greater the affinity of the enzyme for the substrate. Both Km and Vmax affected by environmental conditions such as pH and temperature. The Michaelis–Menten equation is:

$$v0 = \frac{Vm[S]}{Km + [S]}$$

Where:

- v0 : initial velocity.
- Vm: the maximum velocity.
- Km: Michaelis constant.
- [S]: substrate concentration.

A convenient way to verify this equation is to simply remember that v0 = 1/2 Vm when [S] = Km. Therefore, by simple substitution.

3.6.2 Factors that affect enzyme reaction rate

The velocity of an enzyme-catalyzed reaction is affected by a number of factors, including enzyme and substrate concentrations, temperature, pH, ionic strength, and the presence of inhibitors and activators. Some enzymes show an absolute requirement for a particular inorganic ion for activity while others show increased activity when small molecules are included in the reaction medium. These small molecules can play a role in maintaining the conformation of the protein, or they may form an essential component of the active site, or they may form part of the substrate of the enzyme.

In some cases, the activator forms a nearly irreversible association with the enzyme. These non-protein portions of the enzyme are called prosthetic groups. Prosthetic groups are

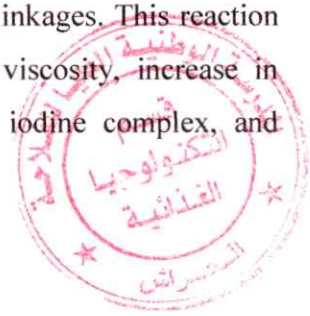
types of cofactors that are tightly bound to enzymes and are required for their activity. They are often organic molecules, such as heme or biotin that are covalently attached to the enzyme's structure.

In most cases, dissociation constants for an enzyme activator complex are within the range of enzyme concentration. Dissociable nonprotein parts of enzymes are categorized as coenzymes. Coenzymes are small organic molecules that are required for the activity of some enzymes. They are not part of the enzyme's structure but are loosely bound to it and can be recycled. They often act as carriers of chemical groups or electrons, and can be derived from vitamins or other nutrients. Examples of coenzymes include NAD+, FAD, coenzyme A, and thiamine pyrophosphate.

3.6.3 Methods of Measurement

A wide variety of methods are available to follow enzyme reactions, including absorbancespectrometry, fluorimetry, manometric methods, titration, isotope measurement, chromatography, mass spectrometry, and viscosity.

An example of using several methods to measure the activity of an enzyme is in the assay of α-amylase activity. α-Amylase cleaves starch at α-1,4 linkages in starch and is an endoenzyme. An endoenzyme cleaves a polymer substrate at internal linkages. This reaction can be followed by a number of methods, including reduction in viscosity, increase in reducing groups upon hydrolysis, reduction in color of the starch iodine complex, and polarimetry.



3.6.4 Applications

3.6.4.1 Specific Applications of Substrate Assays

a- Measurement of Sulfite:

Sulfite is a food additive that can be measured by several techniques, including titration, distillation followed by titration, gas chromatography, and colorimetric analysis. Sulfite also can be specifically oxidized to sulfate by the commercially available enzyme sulfite oxidase (SO):

$$SO^{2-}{}_3 + O2 + H_2O \xrightarrow{SO} SO^{2-}{}_4 + H_2O_2$$

The H_2O_2 product can be measured by several methods including use of the enzyme NADH-peroxidase:

$$H_2O_2 + NADH + H+ \xrightarrow{NADH-peroxidase} 2H_2O + NAD+$$

The amount of sulfite in the system is equal to the NADH oxidized, which is determined by decrease in absorbance at 340 nm. Ascorbic acid can interfere with the assay but can be removed by using ascorbic acid oxidase.

b- Starch/Dextrin Content

Starch and dextrins can be determined by enzymatic hydrolysis using amyloglucosidase, an enzyme that cleaves α-1,4 and α-1,6 bonds of starch, glycogen, and dextrins, liberating glucose. The glucose formed can be subsequently determined enzymatically. Glucose can be determined by colorimetric method, in which glucose is oxidized by glucose oxidase and coupled to a colored dye via reaction of the glucose oxidase product, hydrogen peroxide, with peroxidase. An alternative method of measuring glucose is by coupling hexokinase (HK) and glucose6-phosphate dehydrogenase (G6PDH) reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP+} \xrightarrow{\text{G6PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H+}$$

The amount of NADPH formed is measured by absorbance at 340 nm and is a stoichiometric measure of the glucose originating in the dextrin or starch hydrolyzed by amyloglucosidase. The amount of starch determined by this method is calculated as follows:

$$c = \frac{V\,MW}{\varepsilon\, b\, v 1000} \times \Delta A340$$

Where:

- c = starch in sample solution (g/L).
- V = volume (ml) of reaction mixture.
- MW = molecular weight of starch (because this method measures glucose derived from starch, use 162.1, MWglucose − MWwater).
- ε = absorption coefficient of NADPH at 340 nm (6.3 L mmol−1 cm−1).
- b = light pathlength of cuvette (1 cm).
- v = volume of sample (ml).
- ΔA340 = A340, sample − A340, reagent blank.

This assay sequence can be used to detect the dextrins of corn syrup used to sweeten a fruit juice product.

3.6.4.2 Enzyme Activity Assays

a- Peroxidase Activity

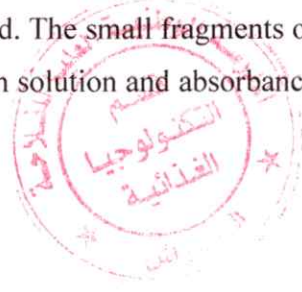
Peroxidase is found in most plant materials and is reasonably stable to heat. A heat treatment that will destroy all peroxidase activity in a plant material is usually considered to be more than adequate to destroy other enzymes and most microbes present. In vegetable processing, therefore, the adequacy of the blanching process can be monitored by following the disappearance of peroxidase activity. Peroxidase catalyzes the oxidation of guaiacol (colorless) in the presence of hydrogen peroxide to form tetraguaiacol (yellow brown) and water. Tetraguaiacol has an absorbance maximum around 450 nm. Increase in absorbance at 450 nm can be used to determine the activity of peroxidase in the reaction mixture.

b- Phosphatase Assay

Alkaline phosphatase is a relatively heat stable enzyme found in raw milk. The thermal stability of alkaline phosphatase in milk is greater than the nonspore forming microbial pathogens present in milk. The phosphatase assay has been applied to dairy products to determine whether pasteurization has been done properly and to detect the addition of raw milk to pasteurized milk. A common phosphatase test is based on the phosphatase-catalyzed hydrolysis of disodium phenyl phosphate liberating phenol. The phenol product is measured colorimetrically after reaction with CQC (2,6-dichloroquinonechloroimide) to form a blue indophenol. The indophenol is extracted into n-butanol and measured at 650 nm.

c- Rennet Activity

Rennet, an extract of bovine stomach, is used as a coagulating agent in cheese manufacture. Most rennet activity tests are based on noting the ability of a preparation to coagulate milk. For example, 12 % non fat dry milk is dispersed in a 10 mM calcium chloride solution and warmed to 35°C. An aliquot of the rennet preparation is added and the time of milk clotting observed visually. The activity of the preparation is calculated in relationship to a standard rennet. As opposed to coagulation ability, rennet preparations can also be evaluated for proteolytic activity by measuring the release of a dye from azocasein (casein to which a dye has been covalently attached). In this assay, the rennet preparation is incubated with 1% azocasein. After the reaction period, the reaction is stopped by addition of trichloroacetic acid. The trichloroacetic acid precipitates the protein that is not hydrolyzed. The small fragments of colored azocasein produced by the hydrolysis of the rennet are left in solution and absorbance read at 345 nm.



3.6.5 Biosensors/Immobilized Enzymes

The use of immobilized enzymes as analytical tools is currently receiving increased attention. An immobilized enzyme in concert with a sensing device is an example of a biosensor. A biosensor is a device comprised of a biological sensing element (e.g., enzyme, antibody, etc.) coupled to a suitable transducer (e.g., optical, electrochemical, etc.). Immobilized enzymes, because of their stability and ease of removal from the reaction, can be used repeatedly, thus eliminating a major cost in enzyme assays. For example for sucrose analysis, invertase, mutarotase, and glucose oxidase are immobilized on membrane.

3.7 Vitamin analysis

3.7.1 Importance of analysis

Vitamin analysis of food and other biological sam1ples has played a critical role in determining animal and human nutritional requirements. Furthermore, accurate food composition information is required to determine dietary intakes to assess diet adequacy and improve human nutrition worldwide.

- Vitamin units: When vitamins are expressed in units of mg or µg per tablet or food serving, it is very easy to grasp how much is present. Vitamins can also be expressed as international units (IU), United States Pharmacopeia (USP) units, and % Daily Value (DV).
- Vitamin assays can be classified as follows:

1. Bioassays involving humans and animals.
2. Microbiological assays making use of protozoan organisms, bacteria, and yeast.
3. Physicochemical assays that include spectrophotometric, fluorometric, chromatographic, enzymatic, immunological, and radiometric methods.

In terms of ease of performance, but not necessarily with regard to accuracy and precision, the three systems follow the reverse order. It is for this reason that bioassays, on a routine basis at least, are limited in their use to those instances in which no satisfactory alternative method is available.

3.7.2 Extraction methods

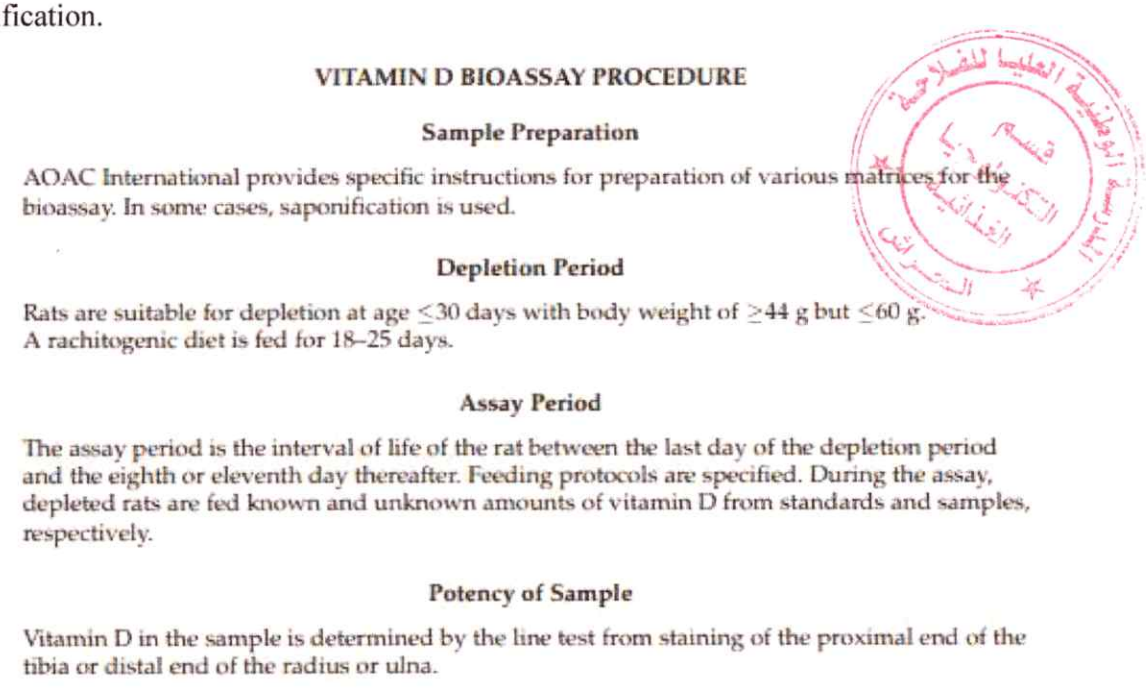
With the exception of some biological feeding studies, vitamin assays in most instances involve the extraction of a vitamin from its biological matrix prior to analysis. This generally includes one or several of the following treatments: heat, acid, alkali, solvents, and enzymes.

In general, extraction procedures are specific for each vitamin and designed to stabilize the vitamin. In some instances, some procedures are applicable to the combined extraction of more than one vitamin, for example, for thiamin and riboflavin as well as some of the fat-soluble vitamins. Typical extraction procedures are as follows:

- Ascorbic acid: Cold extraction with metaphosphoric acid/acetic acid.
- Vitamin B1 and B2: Boiling or autoclaving in acid plus enzyme treatment.
- Niacin: Autoclaving in acid (non cereal products) or alkali (cereal products).
- Folate: Enzyme extraction with α-amylase, protease and γ-glutamylhydrolase (conjugase).
- Vitamins A, E, or D: Organic solvent extraction, saponification, and re-extraction with organic solvents. For unstable vitamins such as these, antioxidants are routinely added to inhibit oxidation. Analysis of fat-soluble vitamins may require saponification, generally either overnight at room temperature or by refluxing at 70 °C.

3.7.3 Bioassay Methods

Outside of vitamin bioavailability studies, bioassays at the present are used only for the analysis of vitamins B12 and D. For the latter, it is the reference standard method of analysis of food materials (AOAC Method 936.14), known as the line test **(Fig.20)**, based on bone calcification.



VITAMIN D BIOASSAY PROCEDURE

Sample Preparation

AOAC International provides specific instructions for preparation of various matrices for the bioassay. In some cases, saponification is used.

Depletion Period

Rats are suitable for depletion at age ≤30 days with body weight of ≥44 g but ≤60 g. A rachitogenic diet is fed for 18–25 days.

Assay Period

The assay period is the interval of life of the rat between the last day of the depletion period and the eighth or eleventh day thereafter. Feeding protocols are specified. During the assay, depleted rats are fed known and unknown amounts of vitamin D from standards and samples, respectively.

Potency of Sample

Vitamin D in the sample is determined by the line test from staining of the proximal end of the tibia or distal end of the radius or ulna.

Figure. 20 The bioassay of vitamin D by the line test, AOAC Method 936.14, 45.3.01

3.7.4 Microbiological Assays

Microbiological assays are limited to the analysis of water-soluble vitamins. The methods are very sensitive and specific for each vitamin.

- **Principle:**

The growth of microorganisms is proportional to their requirement for a specific vitamin. Thus, in microbiological assays the growth of a certain microorganism in an extract of a vitamin-containing sample is compared against the growth of this microorganism in the presence of known quantities of that vitamin. Bacteria, yeast, or protozoans are used as test organisms. Growth can be measured in terms of turbidity, acid production, gravimetry, or by respiration. The microorganisms are specified by ATCC™ numbers and are available from the American Type Culture Collection (ATCC™).

3.7.5 Chemical methods

3.7.5.1 Vitamin E (Tocopherols and Tocotrienols)

Vitamin E is present in foods as eight different compounds: all are 6-hydroxychromans. The vitamin E family is comprised of α-, β-, γ-, and δ-tocopherol, characterized by a saturated side chain of three isoprenoid units and the corresponding unsaturated tocotrienols (α-, β-, γ-, and δ-).

Procedure The vitamin E assay is detailed in **Figure (21)**.

VITAMIN E HPLC ANALYSIS PROCEDURE

Sample Preparation

a. ***General food products***: Add 10 ml of 6% (w/v) pyrogallol in 95% ethanol to sample, mix, and flush with N_2. Heat at 70°C for 10 min with sonication. Add 2 ml of 60% (w/v) KOH solution, mix, and flush with N_2. Digest for 30 min at 70°C. Sonicate 5 min. Cool to room temperature, and add sodium chloride and deionized H_2O. Extract 3X with hexane (containing 0.1% BHT). Combine hexane extracts. Add 0.5 g of anhydrous $MgSO_4$ and mix. Filter through a Millipore filtration apparatus (0.45 µm). Dilute to volume with hexane. Inject sample into HPLC.

b. ***Margarine and vegetable oil spreads***: Add 40 ml of hexane (containing 0.1% BHT) to a 10-g sample and mix. Add 3 g of anhydrous $MgSO_4$, mix, let stand ≥2 hr. Filter and dilute combined filtrate to volume with hexane (0.1% BHT). Inject sample into HPLC.

Chromatography Parameters

Column	Hibar® LiChrosorb Si 60 (4 mm × 250 mm, 5-µm particle size) and LiChromCART® 4-4 guard column packed with LiChrospher® Si 60 (5 µm)
Mobile Phase	Isocratic, 0.85% (v/v) 2-propanol in hexane
Injection Volume	20 µl
Flow	1 ml/min
Detector	Fluorescence, $E_x\lambda = 290$ nm, $E_m\lambda = 330$ nm

(Note: Determine recovery for each food product.)

Figure. 21 Analysis of vitamin E in food products using HPLC.

3.7.5.2 Vitamin C

The vitamin (L-ascorbic acid and L-dehydroascorbic acid) is very susceptible to oxidative deterioration, which is enhanced by high pH and the presence of ferric and cupric ions. For these reasons, the entire analytical procedure needs to be performed at low pH and, if necessary, in the presence of a chelating agent.

❖ **2,6-Dichloroindophenol Titrimetric Method (AOAC Method 967.21, 45.1.14):**

1. Principle. L-ascorbic acid is oxidized to L-dehydroascorbic acid by the oxidation–reduction indicator dye, 2,6-dichloroindophenol. At the endpoint, excess unreduced dye appears rose-pink in acid solution.

2. Procedure. **Figure (22)** outlines the protocol followed for this method. In the presence of significant amounts of ferrous Fe, cuprous Cu, and stannous Sn ions in the biological matrix to be analyzed, it is advisable to include a chelating agent such as ethylenediaminetetraacetic acid (EDTA) with the extraction to avoid overestimation of the ascorbic acid content. The light but distinct rose-pink endpoint should last more than five second to be valid. With colored samples such as red beets or heavily browned products, the endpoint is impossible to detect by human eyes. In such cases it, therefore, needs to be determined by observing the change of transmittance using a spectrophotometer with the wavelength set at 545 nm.

3. Calculations.

$$\boldsymbol{mg\ of\ ascorbic\ acid/g\ or\ ml\ of\ sample} = (\boldsymbol{X} - \boldsymbol{B}) \times (\boldsymbol{F/E}) \times (\boldsymbol{V/Y})$$

Where:

X = average ml for test solution titration.

B = average ml for test blank titration.

F = mg ascorbic acid equivalents to 1.0-ml indophenol standard solution.

E = sample weight (g) or volume (ml).

V = volume of initial test solution.

Y = volume of test solution titrated.

Note. The (V/Y) term represents the dilution factor employed.

VITAMIN C ASSAY PROCEDURE 2,6-DICHLOROINDOPHENOL TITRATION

Sample Preparation

Weigh and extract by homogenizing test sample in metaphosphoric acid-acetic acid solution (*i.e.*, 15 g of HPO_3 and 40 ml of HOAc in 500 ml of deionized H_2O). Filter (and/or centrifuge) sample extract, and dilute appropriately to a final concentration of 10–100 mg of ascorbic acid/100 ml.

Standard Preparation

Weigh 50 mg of USP L-ascorbic acid reference standard and dilute to 50 ml with HPO_3-HOAc extracting solution.

Titration

Titrate three replicates each of the standard (*i.e.*, to determine the concentration of the indophenol solution as mg ascorbic acid equivalents to 1.0 ml of reagent), test sample, and blank with the indophenol reagent (*i.e.*, prepared by dissolving 50 mg of 2,6-dichloroindophenol sodium salt and 42 mg of $NaHCO_3$ to 200 ml with deionized H_2O) to a light but distinctive rose pink endpoint lasting ≥5 sec.

Figure 22: Analysis of vitamin C by the 2,6-dichloroindophenol titration, AOAC Method 967.21, 45.1.14

3.7.6 COMPARISON OF METHODS

Each type of method has its advantages and disadvantages. In selecting a certain method of analysis for a particular vitamin or vitamins, a number of factors need to be considered, some of which are listed below:

1. Method accuracy and precision.
2. The need for bioavailability information.
3. Time and instrumentation requirements.
4. Personnel requirements.
5. The type of biological matrix to be analyzed.
6. The number of samples to be analyzed.
7. Regulatory requirements – Must official AOAC International methods be used?

Part IV.

4 Toxicology

4.1 Fundamentals of toxicology

There are three so-called laws that underlie the science of toxicology. Paracelsus is usually considered to be the father of toxicology, having formulated the first law, which states that the dose makes the poison. The second law concerns the specificity of toxic effects of individual chemicals, a specificity due to the unique chemical structure of the agent and the laws of biology that govern the response. The third law is that humans are animals and that therefore the study of animals can provide useful insight into effects in humans.

4.2 Legislation

Knowledge of government regulations relevant to the chemical analysis of foods is extremely important to persons working in the food industry. Federal laws and regulations reinforce the efforts of the food industry to provide wholesome foods, to inform consumers about the nutritional composition of foods, and to eliminate economic frauds. In some cases, they dictate what ingredients a food must contain, what must be tested, and the procedures used to analyze foods for safety factors and quality attributes.

The US federal regulations related to the composition of foods are decribed below:

- **Federal Food, Drug, and Cosmetic Act of 1938:** The Federal Food, Drug, and Cosmetic (FD&C) Act of 1938 was intended to assure consumers that foods are safe and wholesome, produced under sanitary conditions, and packaged and labeled truthfully. This law, which broadened the scope of the Food and Drug Act of 1906, further defined and set regulations on adulterated and misbranded foods.
- **Environmental Protection Agency (EPA):** The Miller Pesticide Amendment was added in 1954 to specify the acceptable amount of pesticide residues on fresh fruits, vegetables, and other raw agricultural products when they enter the marketplace.
- **The Food Additives Amendment (FAA)** enacted in 1958 was designed to protect the health of consumers by requiring a food additive to be proven safe before addition to a food and to permit the food industry to use food additives that are safe at the intended level of use.
- **The Color Additives Amendment (CAA)** of 1960 defines color additives, sets rules for both certified and uncertified colors, provides for the approval of color additives that must be certified or are exempt from certification, and empowers the FDA to list color additives for specific uses and set quantity limitations.

- **The Nutrition Labeling and Education (NLEA)** Act of 1990, made nutrition labeling mandatory on most food products.
- **The Dietary Supplement Health and Education** Act (1994) **(DSHEA)** changed the definition and regulations for dietary supplements.
- **Hazard Analysis Critical Control Point (HACCP)** is an internationally recognized systematic approach that is used to prevent and/or controlmicrobial, chemical, and physical hazards within the food supply. The "farm to the fork" approach was originally designed to be used by the food processing industry to produce zero defect (no hazard) food for astronauts to consume on space flights.

4.3 Identification of major food toxins

The severity of an adverse effect associated with a food hazard is usually directly related to the dose. In many cases there is a threshold level (tolerance level) below which no adverse effects are observed. The US Environmental Protection Agency (EPA) establishes these tolerance levels, and the Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) enforces them. However, food safety incidents, microbial and chemical in nature, continue to occur. Pesticide residues, mycotoxins, veterinary drug residues, some food additives, food adulterants, packaging hazardous chemicals, and environmental contaminants are of concern.

The **Rapid Alert System for Food and Feed (RASFF)** reported that in the period between July 2003 and June 2007 a total of 12,641 alert notifications categorized as follows: chemical (44 %), mycotoxins (29 %), microbial (17 %), and other hazards (10 %) **(Fig.23)**. Within the chemical category, the most frequently reported hazards include allergens (e.g., histamine and sulfite), heavy metals (e.g., mercury, lead, and cadmium), pesticides (e.g., omethoate, dimethoate, and isophenfos-methyl), and veterinary drugs (e.g., β-lactam, nitrofurans, sulfonamide, and chloramphenicol). Microbial contaminants include molds, viruses, and bacteria.Examples of current and emerging chemical hazards include fraud and food adulterants (e.g., melamine), packaging chemicals (e.g., bisphenol A and 4-methyl benzophenone), degradation metabolites (e.g., acrylamide and furan), and other chemical contaminants (e.g., 3-monochloropropane-1,2-diol, benzene, and perchlorate). Another category that can be of concern includes the genetically modified organisms (GMO) and their products. Introduction and usages of GMO in food products resulted in the development of legal requirements of safety and labeling.Given the extent of concerns cited above, there is a

strong need for adequate and reliable methods of detection and analysis to ensure food quality, safety, and fair trade.

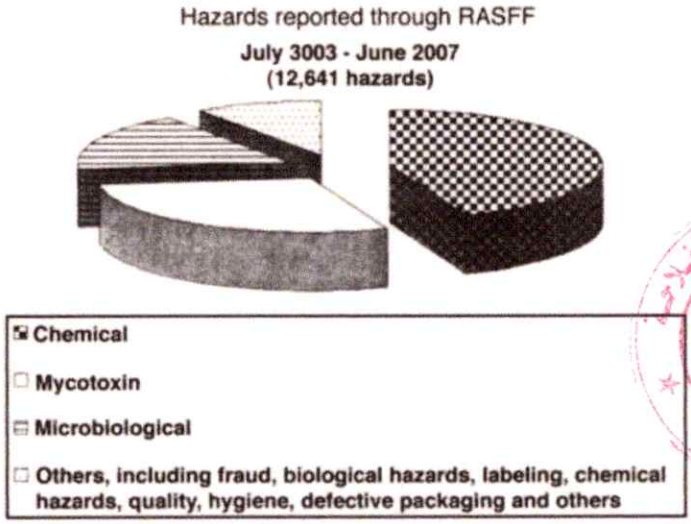


Figure 23: Categorized food chain hazards as reported through the Rapid Alert System for Food and Feed (RASEF) in the period of July 2003 to June 2007.

4.3.1 Antinutritional factors

Antinutritional factors (ANFs) are deleterious compounds present in the grain which interfere with the absorption of biomolecules and hamper their bioavailability to the human beings and monogastric animals.

Several types of anti-nutritional factors with toxic potential have been measured in foods and shown to be heat-stable or heat-labile. These factors include saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, antivitamin factors, metal binding ingredients, goitrogens, etc. Nutrition-related problems and harmful effects to human health are raised by these factors, which are present in the seeds of cereals and legumes. **Figure (24)** shows a brief overview of the adverse effects of key anti-nutrients that are present in foods.

Wheat is an important staple cereal providing major source of energy and protein across the world. However, there are several antinutritional factors present in wheat, such as phytate, protease inhibitor, tannins, lectins, alkaloids, oxalate, etc. Phytate, being the most important among all, reduces the bioavailability of micronutrients such as iron and zinc.

Pulses are also rich in ANFs. ANF in pulses includes amylose inhibitor, trypsin inhibitor, saponins, agglutinins, etc. Among these inhibitors, trypsin inhibitor is of major concern as it

reduces the availability of proteins by reducing the digestibility of specific amino acids. To reduce ANF in pulses, generally, pulses are heat-treated or cooked with water.

phytic acid or phytates (22.5 mg/g) and trypsin inhibitors (2.88 TIU/mg protein) present as the dominant antinutritional factors in HM. Various approaches including traditional and molecular breeding and genetic engineering have been deployed to reduce antinutritional factors in the grains. Here, we discuss various ways and means to reduce their inhibitory effect on bioavailability of micronutrients.

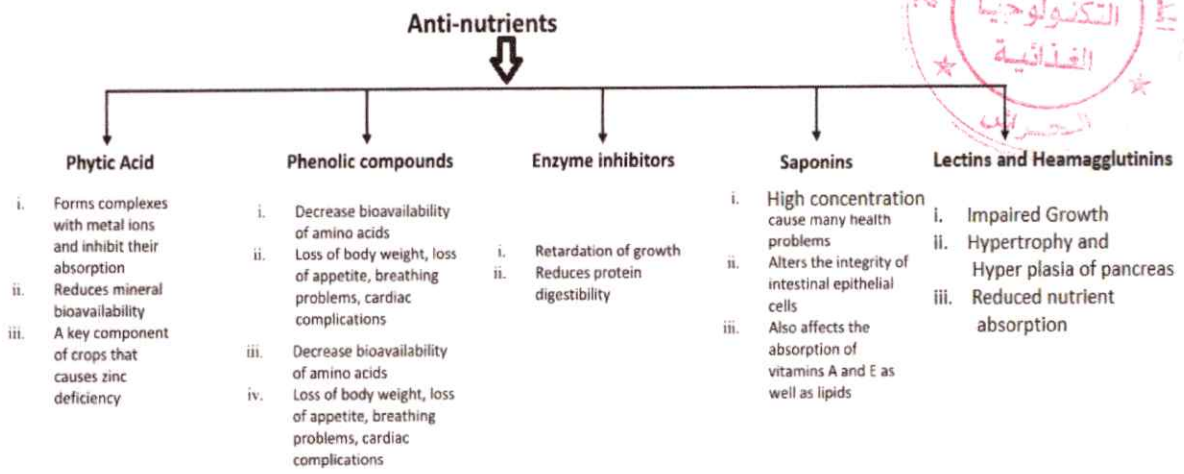


Figure 24: A brief overview of the adverse effects of key anti-nutrients. (Samtiya et al., 2020)

❖ **Strategies used to reduce food levels of anti-nutrients:**

Besides their property of reduction of various minerals and nutrients, these anti-nutrients could cause toxicity when present in higher amounts in the diet. Due to these reasons, reduction in the anti-nutritional content of foods is of great interest. Different traditional methods and technological processing ways such as soaking, milling, debranning, roasting, cooking, germination and fermentation have been used for reducing these anti-nutritional components in foods.

4.3.2 Heavy metals

Heavy metals (HMs) naturally occur in the environment and are vital for survival, but they may become hazardous when they accumulate in organisms. A few of the most frequent heavy metals that contaminate the environment include mercury, cadmium, arsenic, chromium, nickel, copper, and lead. The poisoning can happen if you eat or drink something

tainted with heavy metals or if you breathe in contaminated dust or fumes. **Table (7)** shows the sources, adverse effects, and significance of toxic HMs.

You might get heavy metal poisoning if you:

- Work in a factory that uses heavy metals.
- Breathe in old lead paint dust when you fix up your home.
- Eat fish caught in an area with high levels of mercury.
- Use herbal medicines that have heavy metals in them.
- Use dinnerware that hasn't been coated well enough to prevent heavy metals from contaminating food.
- Drink water contaminated with heavy metal.

Table 7: Sources of HMs in the environment Sodhi et al. (2022)

HM	Sources	Adverse effects of the HMs	Significance of Heavy metal
Cu^{2+}	**Fertilizers, Agricultural fungicides**, electroplating, algicides	Wilson's disease, headaches, nausea, dizziness due to long-term exposure	An essential nutrient, Helps in the formation of RBC with Iron. Helps in bone formation, blood vessels, and the functioning of the immune system.
Cd^{2+}	Agricultural fungicides, **electroplating**, algicides, Cd & Ni batteries, welding	The cumulative toxin, carcinogenic, neural problems, and kidney failure. In plants, leads to tissue death	No significant function in the human body. Transported in the blood bound to metallothionein.
Cr^{6+}	Chrome plating, **paints**, dyes, ceramics	Lung cancer, pulmonary illness	Helps in the breakdown of carbohydrates and fats. Stimulate the synthesis of cholesterol and fatty acids. Helps in the action of insulin and also in the breakdown of glucose.
Ni^{2+}	Chrome plating, Cd & Ni batteries	Allergic contact dermatitis. oxidative stress, nutritional imbalance in plants	A micronutrient important for the proper functioning of the human body increases hormonal activity and helps in lipid metabolism. A micronutrient in plants too and helps in nitrogen fixation and urea metabolism.
Zn^{2+}	Refineries, metal plating, plumbing, brass manufacture	Headaches, nausea, dizziness due to long-term exposure, Night blindness	Zinc is important for the immune system to function properly and plays an important role in cell growth, cell division, also in wound healing. Helps in the breakdown of carbohydrates which is an important dietary nutrient. In zinc-deficient plants, chlorophyll synthesis is reduced significantly.
As^{3+}	Mining, smelting, fossil fuels, dietary intake (cereals, poultry, fish, and dairy products)	Carcinogen leading to lung cancer and skin cancer, hyperpigmentation, keratosis	In industries as an alloying agent for the smelting, used in textiles and paper industries, pesticides, feed additives but no significant function in the human body

Anti-inflammatory compounds, natural and synthetic antioxidants, glutamate protectors, and ATP/ADP ratio protectors have been used to decrease heavy metal toxicity. **Figure (25)** present diagrammatic explanation of heavy metal toxicity treatment by natural bioactive molecules.

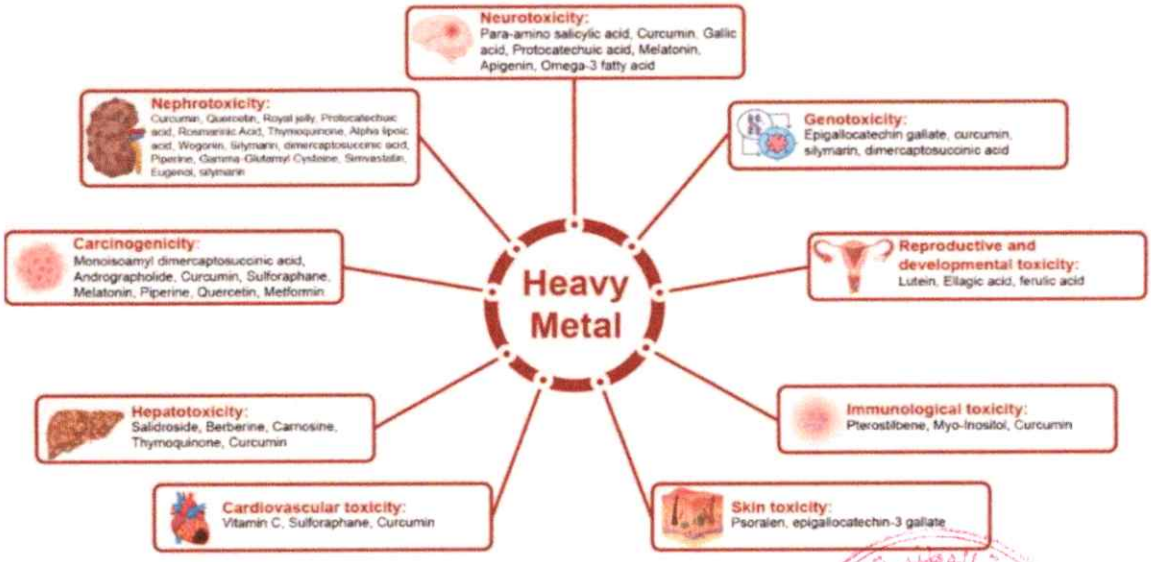


Figure 25: Diagrammatic explanation of heavy metal toxicity treatment by natural bioactive molecules (Mitra et al., 2022)

4.3.3 Mycotoxins

Mycotoxins are toxic metabolises produced by fungi, especially by saprophytic moulds growing on foodstuffs or animal feeds. The Greek word "mykes" meaning "fungi" and the Latin word "toxicum" meaning "poison" are the origin of the word mycotoxin. It is now well established that mycotoxicoses (the diseases caused by mycotoxins) have been responsible for major epidemics in man and animals at least during recent historic times. The most important have been ergotism, which killed thousands of people in Europe in the last thousand years, alimentary toxic aleukia (ATA) which was responsible for the death of many thousands of people in the USSR in the 1940s; stachybotryotoxicosis, which killed tens of thousands of horses and cattle in the USSR in the 1930s; and aflatoxicosis, which killed 100,000 young turkeys in England in 1960 and has caused death and disease in many other animals, and perhaps man as well. Each of these diseases is now known to have been caused by growth of specific moulds which produced one or more potent toxins, usually in one specific kind of commodity or feed.

It is important to distinguish between the effects of bacterial toxins and mycotoxins. The classic bacterial toxins are proteins, which produce characteristic symptoms in only a few hours, as the human body recognises them, and produces antibody mediated reactions to them. Fungal toxins on the other hand, are almost all low molecular weight chemical compounds which are not detected by antigens, and hence produce no obvious symptoms. Mycotoxins are insidious poisons (chronic effect).

Of the approximate 400 compounds identified as mycotoxins, 30 receive special attention and are considered a threat to human and animal health. The most important mycotoxins are **(Fig.26)**:

- **Aflatoxins (AFs),** represented mainly by aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1)
- **Ochratoxins (OTs),** represented mainly by ochratoxin A (OTA))
- **Fumonisins (FBs),** represented mainly by fumonisins B1 (FB1), B2 (FB2), and B3 (FB3))
- **Trichothecenes (TCs),** with type A represented by HT-2 toxin (HT2) and T-2 toxin (T2), and type B represented mainly by deoxynivalenol (DON)
- **Zearalenone (ZEN)**
- **The emerging Fusarium mycotoxins** (fusaproliferin (FP), moniliformin (MON), beauvericin (BEA), NX-2 toxin, and enniatins (ENNs)
- **Ergot alkaloids (EAs)**
- **Alternaria toxins (ATs),** such as altenuene (ALT), alternariol (AOH), alternariol methyl ether (AME), altertoxin (ALTs), and tenuazonic acid (TeA)
- **Patulin (PAT)**

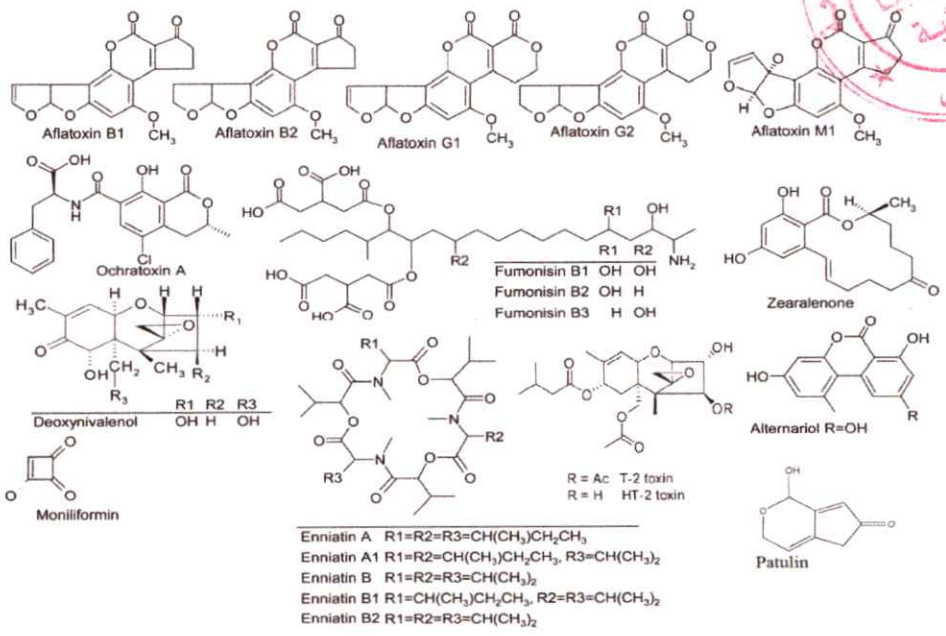


Figure 26: Structure of the most important mycotoxins. Image Source: https://www.mdpi.com/2304-8158/9/2/137/htm

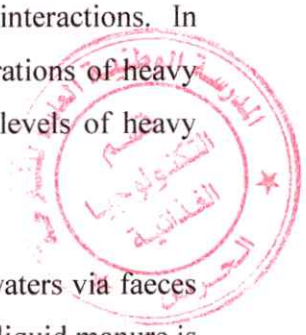
Many mycotoxins, such as the aflatoxins G1, G2, B1, B2, or ochratoxin A, have been highlighted as immunosuppressive, carcinogenic, neurologically toxic, and hepatotoxic.

Aspergillus mould can cause lung infections such as aspergillosis. Considering the great risk to consumer health, accurate testing methods are of great importance.

Mycotoxins are among the most prominent and dangerous toxins associated with food safety. They attract worldwide attention because they are important contaminants with a multitude of effects on both human and animal health. Food can be safe and of high quality if Hazard Analysis and Critical Control Points (HACCP) is implemented across the food chain from farm to fork and at all stages of food handling. Prevention is an important strategy in the fight against mycotoxins and should be applied in the pre-harvest stages, in raw materials and processed foods. Detoxification methods can also be applied without affecting the smell/taste characteristics of the foods.

4.3.4 Soil contaminants

Soil properties are crucial in food production, and heavy metal pollution of this critical resource, as well as their subsequent absorption and bioaccumulation in food crops, poses substantial environmental and health concerns, especially in poor countries. Heavy metal concentrations are influenced by soil type, plant genotype, and their interactions. In comparison to organic manure, mineral fertilizers contain increased concentrations of heavy metals; as a consequence, the use of mineral fertilizers leads in increased levels of heavy metal pollution in soil.



4.3.4.1 Medicinal Residues in Soil and Surface Waters

In agriculture, veterinary medicinal products can enter soil and surface waters via faeces or sewage sludge. Up to 75 % of any applied antibiotic is excreted. However, liquid manure is usually stored prior to application to agricultural soils and during storage, the amount of the medicinal product is reduced by degradation.

The occurrence of medicinal residues in surface water is well documented in the scientific literature. Various studies have investigated the concentrations of active pharmaceutical ingredients and metabolites in wastewater, surface water and groundwater, such as antibiotics, anti-epileptics, lipid-lowering agents or antiphlogistics (pain and rheumatism drugs). These relate to residues of both veterinary and human medicines.

4.3.4.2 Nitrate contamination of soil and water

Human-induced changes in the global nitrogen (N) cycle have resulted in more than doubling the amount of reactive N in the environment, posing significant threats to ecosystem functions and human health. The world's rising population and subsequently increasing demands for water and food security have led to more chemical N fertilizers being employed,

and more organic N sources such as animal manures and sewage sludge many-fold. This has resulted in a rising amount of N in terrestrial and aquatic environments, mainly in the form of nitrate (NO_3). Nitrate contamination of surface and groundwater has raised serious concerns for ecosystem functions and people's health, and is linked to a number of United Nations' stipulated sustainable development goals (SDGs). Excess NO_3 in groundwater causes a variety of diseases in humans and animals but especially of concern are conditions affecting human infants, who suffer more and when they are exposed to higher NO_3 in drinking water.

4.3.5 Authorized and unauthorized additives

4.3.5.1 Definitions:

- Food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities (Codex Alimentarius Procedural Manual).
- Acceptable Daily Intake (ADI) is an estimate by the Joint FAO/WHO **Expert Committee on Food Additives (JECFA)** of the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk. Acceptable Daily Intake "Not Specified" (NS) is a term applicable to a food substance of very low toxicity.
- Maximum Use Level of an additive is the highest concentration of the additive determined to be functionally effective in a food or food category and agreed to be safe by the Codex Alimentarius Commission. It is generally expressed as mg additive/kg of food.

4.3.5.2 General principles for the use of food additives

The use of food additives in conformance with the Standard requires adherence to all the principles set forth in Sections below.

4.3.5.2.1 Food Additive Safety

a) Only those food additives shall be endorsed and included in this Standard that, so far as can be judged on the evidence presently available from JECFA, present no appreciable health risk to consumers at the use levels proposed.

b) The inclusion of a food additive in this Standard shall have taken into account any ADI, or equivalent safety assessment established for the additive by JECFA and its probable daily intake from all food sources. Where the food additive is to be used in foods eaten by special groups of consumers (e.g. diabetics, those on special medical diets, sick individuals on formulated liquid diets), account shall be taken of the probable daily intake of the food additive by those consumers.

c) The quantity of an additive added to food is at or below the maximum use level and is the lowest level necessary to achieve the intended technical effect.

4.3.5.2.2 Justification for the Use of Additives

The use of food additives is justified only when such use has an advantage, does not present an appreciable health risk to consumers, does not mislead the consumer, and serves one or more of the technological functions set out by Codex and the needs set out from (a) through (d) below, and only where these objectives cannot be achieved by other means that are economically and technologically practicable:

a) To preserve the nutritional quality of the food; an intentional reduction in the nutritional quality of a food would be justified in the circumstances dealt with in sub-paragraph (b) and also in other circumstances where the food does not constitute a significant item in a normal diet;

b) To provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs;

c) To enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that this does not change the nature, substance or quality of the food so as to deceive the consumer;

d) To provide aids in the manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of

the use of faulty raw materials or of undesirable (including unhygienic) practices or techniques during the course of any of these activities.

4.3.5.2.3 Good Manufacturing Practice (GMP)

All food additives subject to the provisions of this Standard shall be used under conditions of good manufacturing practice, which include the following:

a) The quantity of the additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect;

b) The quantity of the additive that becomes a component of food as a result of its use in the manufacturing, processing or packaging of a food and which is not intended to accomplish any physical, or other technical effect in the food itself, is reduced to the extent reasonably possible; and,

c) The additive is of appropriate food grade quality and is prepared and handled in the same way as a food ingredient.

4.3.5.2.4 Specifications for the Identity and Purity of Food Additives

Food additives used in accordance with this Standard should be of appropriate food grade quality and should at all times conform with the applicable Specifications of Identity and Purity recommended by the Codex Alimentarius Commission.

4.3.5.3 Types of Food Additives

The types of food additives help the manufacturer to synthesize and understand the type of additives that could perform specific technological functions in the desired product **(Fig.27)**. The types of food additives are also based on the origin of additives (natural and synthetic).

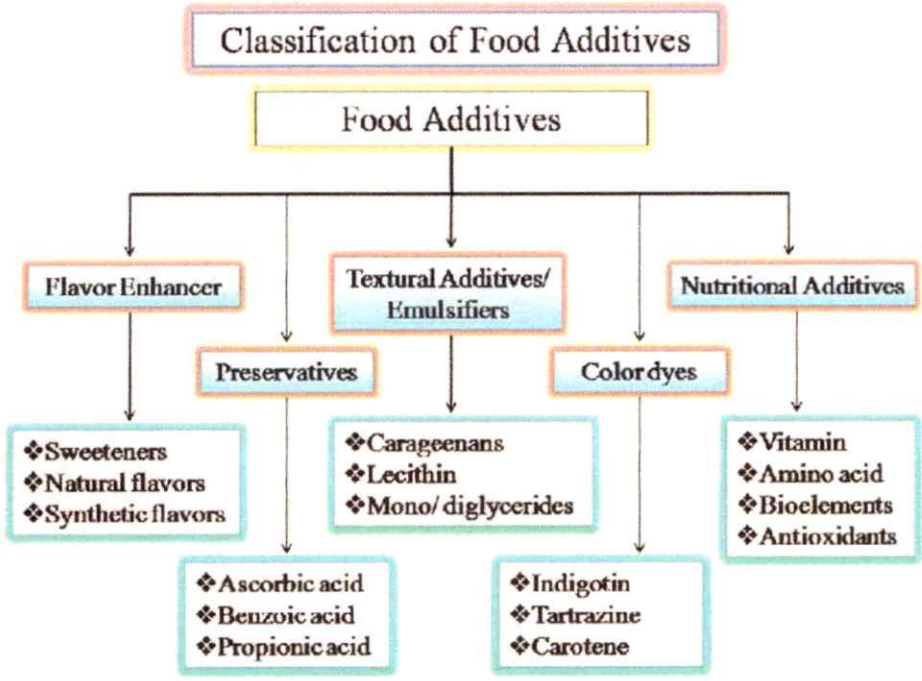


Figure 27: Classification of food additives. Kaur et al., (2023)

4.3.5.4 E- Numbers (International Numbering System) for Food Additives

The Additives which are proclaimed to be safe for usage as per Codex are given 'E-numbers'. These E-numbers specify the identity of a particular additive, which can be later used for labeling of pre-packaged processed food **(Table 8)**. The Identification number or E-number consists of three or four digits such as 100 for Curcumin (Color) and 1000 for Cholic acid (Emulsifier). Sometimes the number is accompanied by an alphabet such as 150a which is given for Caramel –I (color), 150b for Caramel – II.

Table 8: E- Numbers (International Numbering System) for Food Additives

E-Numbers	Food Additives
E100-E199	Colours
E200-E299	Preservatives
E300-E399	Anti-oxidants and Acidity Regulators
E400-E499	Thickeners, Stabilizers and Emulsifier
E500-E599	Anticaking Agents
E600-E699	Flavour Enhancers
E700-E799	Antibiotics
E900-E999	Glazing Agents and Sweeteners
E1000-E1599	Additional Chemicals

4.3.6 Packaging and pesticide residues

4.3.6.1 Packaging residue

Food packaging is manufactured from a range of materials including glass, paper/ paperboard, a variety of plastics, and metals such as aluminium and steel. The bulk packaging material is often modified due to the use of adhesives, protective coatings and printing inks, for example. Several thousand chemicals are used in the manufacture of food packaging and other materials that come into contact with food during its production and processing. For example, more than 3200 food contact substances are listed in the US Code of Federal Regulations. Chemicals used in the production of food contact materials include solvents, monomers, cross-linking agents, catalysts, plasticisers, and antioxidants/ stabilisers. The EU is currently evaluating the **FCM (Food contact materials)** legislation in order to harmonise regulations across the European Union. The summary of the current packaging legislation can be found **in (Figure.28)** below.

The scientific principles that apply to the risk assessment of other chemicals in food, such as food additives, contaminants and processing aids, also apply to food packaging chemicals **(FSANZ, Food Standards Australia New Zealand, 2017)**. The core principle in food chemical risk assessment is that risk is a function of both the intrinsic hazard characteristics of the chemical (i.e. its toxicological properties) and dietary exposure to the chemical from consuming food and beverages.

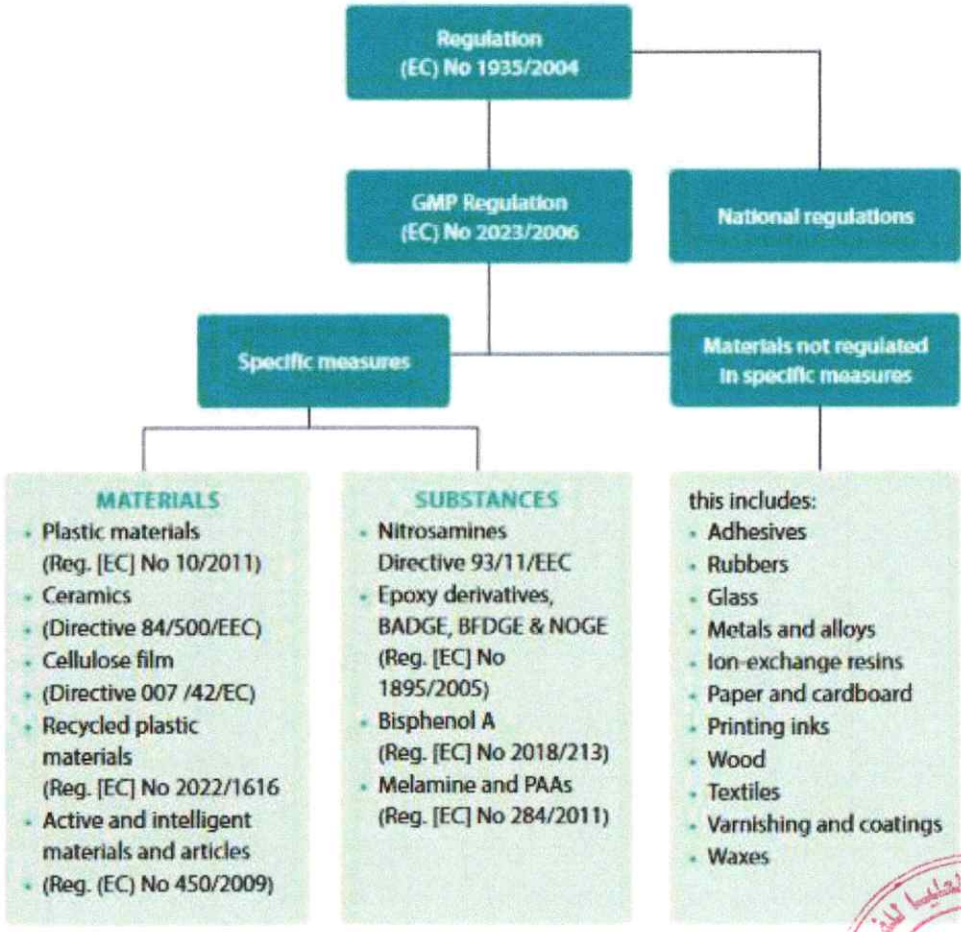


Figure 28: Food packaging guideline|. European Commission Regulation (Reg. EC) Good Manufacturing practice (GMP). (IFS., 2024)

The interaction of food packaging with the food can be described using the following **Figure (29)**:

- Permeation is the exchange of substances from the outside to the inside of a package. This is for example, when solvents of printing ink, gas or moisture enter from outside the packaging and into the food, compromising the protective function of the packaging. This phenomenon can harm food quality and shelf life in packaged foods, as well as transfer harmful substances.
- Sorption is the exchange of food components such as flavours, lipids, and moisture into the packaging material. This can cause changes in flavour and odour of the food. Furthermore, food components that migrate into plastic (e.g. fat) can increase the

mobility of plastic components, accelerating the migration from the food packaging to food.

- Scalping is the escape of food components outside of the packaging, resulting in loss of aroma and a decrease in the organoleptic quality of the food.
- Migration occurs when chemicals and/or components pass from the packaging and into the food. Since these particles can be hazardous to human health, migration is regulated in most countries for this reason.

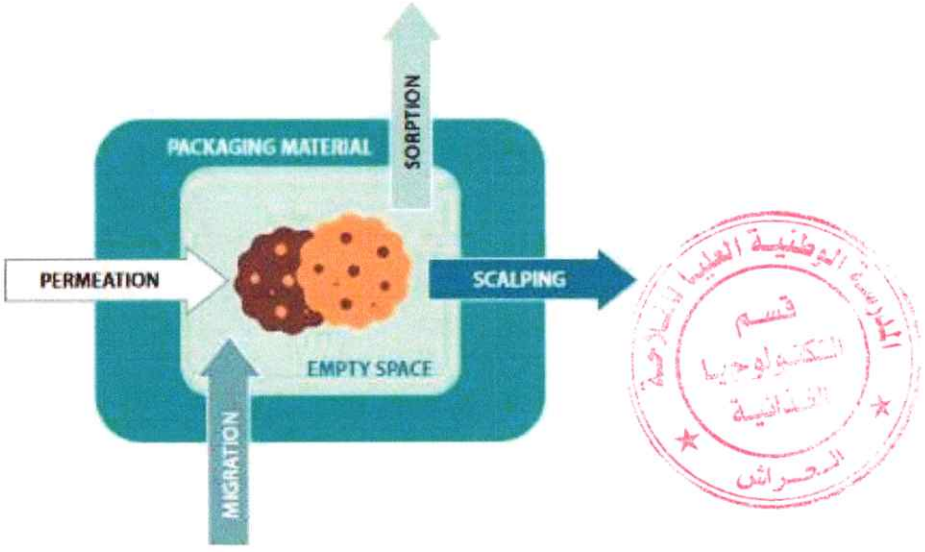


Figure 29: Interaction between food packaging and food. IFS (2024)

4.3.6.2 Pesticide residue

The **Environmental Protection Agency (EPA)** defines pesticide as any substance or mixture of substances intended to prevent, destroy, repel, or mitigate any pest. Pesticides may also be described as any physical, chemical, or biological agent that will kill an undesirable plant or animal pest.

Pesticides are widely used in agriculture to control pests, plant diseases and weeds. They are a necessary tool to provide high crop yields and high quality of food products. However, pesticides are developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health. Despite the regulations, serious concerns about health risks raised from successive exposure to these chemicals, or from their residues inhaled through air or ingested through food and water. Yet, considerable amounts of harmful

pesticide residues often remain in the harvested fruits, becoming a permanent danger to the consumer and creating health hazards ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption.

Actually, the presence of pesticide residues in food receives more worldwide attention and the register and set of the Maximum Residue Limits (MRLs) is part of control authorities' responsibilities. These limits are established either by FAO and European Commission Union (EU) in order to protect the environment and particularly consumer health by minimizing pesticide concentration in fruits and vegetables. Pesticides banned by the EU because of harm and danger are still in our food or environment. Many EU countries continue to allow their use **(Table 9)**.

MRL is the maximum concentration of a possible residue on crop or food commodity resulting from the use of pesticides and is expressed in mg/kg of the commodity. ADI (Acceptable Daily Intake) is an estimate of the amount of a chemical in food that can be ingested daily over a lifetime by humans without appreciable health risk. The concept of the ADI has been developed principally by WHO and FAO for additives to foodstuffs, residues of pesticides and veterinary drugs in foods. MRLs are set 100 times below ADI.

Most pesticides break down with exposure to the weather elements. Microbial activity in the plant, soil and environment also reduces or eliminates residues. In the process of cleaning, peeling and cooking of vegetables, fruits and food grains most pesticide residues are removed and broken-up. The process of digestion and blood cleansing also removes the residues from our body.

Various methods to assess the residual pesticide content have been developed like gas chromatography, two single-residue methods, spectrophotometry, high performance liquid chromatography, spectrophotometry, and different extraction processes. Pesticide inspection is also done by diagnosing of different body fluids like blood, urine, serum, breast milk, semen and also toxicological studies on cell line with the development of different biomarkers.

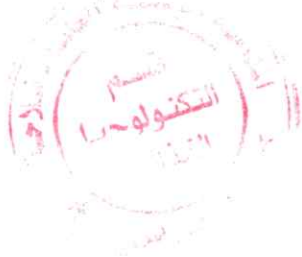


Table 9: Banned pesticides still widely used in many EU countries (Svrtan & Dalhuisen, 2023).

Active substance	Toxicity - reason for banning
1,3-Dichloropropene	Potential concerns for groundwater, non-target arthropods (including bees), birds and mammals, and soil organisms. Potential to act as endocrine disruptor.
Asulam sodium	Risk for consumers, risk to birds and non-targeted terrestrial plants. Acute risk to wild mammals.
Chlorothalonil	Poses the high risk to amphibians and fish. Causes contamination of groundwater by its metabolites. Identified risk for bees. A genotoxicity risk for consumers.
Chlorpyrifos	Genotoxicity and developmental neurotoxicity. Potentially toxic for reproduction.
Chlorpyrifos-methyl	Development and adverse neurodevelopmental outcomes in children. Potentially toxic for reproduction.
Clothianidin	High acute risks for honeybees, solitary bees and bumblebees.
Diquat	Identified high risk to workers, bystanders and residents; high risk to birds.
Imidacloprid	High acute risks for bees.
Linuron	Toxic for reproduction, category 1B and carcinogen, category 2. Poses high risk for children if exposure occures, and for workers operating handheld sprayers, even with the use of the protective equipment. A high risk to birds and wild mammals, non-target arthropods and non-target soil macro-organisms is identified.
Mancozeb	Toxic for reproduction, category 1B. Endocrine-disrupting properties for humans and non-target organisms.
Streptomycin	The approvals of antibiotics for non-medical purposes in agriculture exacerbate the problem of antibiotic resistance.
Thiacloprid	Toxic for reproduction, category 1B and carcinogen, category 2. Metabolites of thiacloprid hold carcinogenic properties and contaminate groundwater.
Thiamethoxam	High acute risks for honeybees, solitary bees and bumblebees.
Thiram	High acute risk to consumers and to workers applying the thiram. Water treatment processes of thiram-containing surface and groundwater result with toxic metabolites. High risk to birds and mammals. High risk to aquatic organisms from exposure to metabolites. Endocrine-disrupting properties.

4.3.7 Extraneous materials

Terms used by AOAC International (AOAC Method 970.66) to classify or characterize various types of extraneous materials are defined as follows.

Extraneous Materials: Any foreign matter in product associated with objectionable conditions or practices in production, storage, or distribution; included are various classes of filth, decomposed material (decayed tissues due to parasitic or nonparasitic causes), and miscellaneous matter such as sand and soil, glass, rust, or other foreign substances. Bacterial counts are not included.

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

Heavy Filth: Heavier material separated from products by sedimentation based on different densities of filth, food particles, and immersion liquids. Examples of such filth are sand, soil, insect and rodent excreta pellets and pellet fragments, and some animal excreta pellets.

Light Filth: Lighter filth particles that are oleophilic and are separated from product by floating them in an oil–aqueous liquid mixture. Examples are insect fragments, whole insects, rodent hairs and fragments, and feather barbules.

Sieved Filth: Filth particles of specific size ranges separated quantitatively from product by use of selected sieve mesh sizes.

The AACC International (formerly American Association of Cereal Chemists) publishes a methods book that includes a section on extraneous matter, containing descriptive material helpful in identifying insect and rodent contaminants. Several microscopic and radiographic illustrations are provided by the AACC International as authentic reference materials to help analysts to identify filth.

There are various laboratory methods for separating (isolating) extraneous materials from foods and for identifying and enumerating them. The FDA and the AOAC International have published reference articles, books, and methods on analysis of extraneous materials. The most authoritative source, and that generally considered official by the FDA, is the Official Methods of Analysis of AOAC International, Chap. 16, "Extraneous Materials: Isolation".

Part V

5 .Analysis of Food Contaminants, Residues, and Chemical Constituents of Concern

The food chain that starts with farmers and ends with consumers can be complex, involving multiple stages of production and distribution (planting, harvesting, breeding, transporting, storing, importing, processing, packaging, distributing to retail markets, and shelf storing). Various practices can be employed at each stage in the food chain, which may include pesticide treatment, agricultural bioengineering, veterinary drug administration, environmental and storage conditions, processing applications, economic gain practices, use of food additives, choice of packaging material, etc. Each of these practices can play a major role in food quality and safety, due to the possibility of contamination with or introduction (intentionally and nonintentionally) of hazardous substances or constituents. Legislation and regulation to ensure food quality and safety are in place and continue to develop to protect the stakeholders, namely farmers, consumers, and industry.

5.1 Choice of Analytical Method

The choice of analytical method for the detection and determination of food hazards must take into account not only the complexity of the food matrix, but also the characteristics of the analyte such as polarity, hydrophobicity, volatility, thermal stability, and chemical reactivity. Methods for the analysis of food hazards can be either qualitative, semiquantitative, or quantitative. A list of analytical methods for pesticide, mycotoxin, and veterinary drug residues is given in **(Table10)**.

5.2 Qualitative and semiquantitative methods

Qualitative and semiquantitative methods, also known as screening methods, are usually used to assay a large number of samples for the presence of one or more contaminants belonging to the same family (e.g., antibiotic residues) in a relatively short time. While qualitative methods detect the presence of certain contaminants, semiquantitative methods provide an estimate of the concentration of a detected contaminant or residue. The principal benefits of these methods are their low cost, relative speed, and simplicity.

5.3 Quantitative Methods

For the quantitative analysis of chemical food contaminants and residues, gas chromatography (CG) and high-performance liquid chromatography (HPLC) are the two main analytical methodologies employed. As compared to HPLC, GC provides better separation efficiency and has been traditionally combined with more selective detectors for the analysis of food contaminants and residues. The combination of GC with mass spectrometry (MS), and the availability of relatively affordable benchtop GCMS instruments, gave preference to GC analysis for multicomponent contaminant and residue analysis, in spite of having to derivatize polar analytes. However, thermally labile and/or large analytes that cannot be easily volatilized, such as mycotoxins, polar pesticides, and most of the veterinary drug residues, currently must be analyzed using HPLC. In recent years, major advances in HPLC-mass spectrometry (LC-MS) have facilitated direct, selective, and sensitive analysis of the polar analytes. For example, LC-MS is gradually replacing microbial and immunochemical methods used for the analysis of veterinary drugs. Pesticides, antibiotics, and mycotoxins are typical examples of contaminants that can be rapidly and efficiently determined using immunoassays. Of the various immunoassay techniques, enzyme-linked immunosorbent assays (ELISA).

Table 10: Summary of analyses for pesticide, mycotoxin, and antibiotic residues in foods

Contaminant	*Quantitative*		*Semiquantitative or Qualitative (Screening Methods)*
Pesticides	Multiresidue (MRMs) GC (mostly) HPLC	Single-residue (SRMs) GC (mostly) HPLC Immunoassay	TLC Enzyme inhibition Immunoassay
Mycotoxins	HPLC (mostly) GC Capillary electrophoresis Immunoassays		TLC Immunoassay
Antibiotics	HPLC (mostly) GC Immunoassays		Microbial growth inhibition Receptor assays Enzyme substrate assays Immunoassays

HPLC, high-performance liquid chromatography; *GC*, gas chromatography; *TLC*, thin-layer chromatography.

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