COURSE CODE: *BCH 318*

COURSE TITLE: *Nutrition, Food Science and Technology*

NUMBER OF UNITS: *2 Units*

COURSE DURATION: *Two hours per week*

COURSE DETAILS:

Course Coordinator: MR. AYODELE Peter Folorunsho
Email:

COURSE CONTENT:

Food constituents. An introduction to the theory and application of physical and chemical methods for determining the constituents of food; processing, preservation and storage of traditional foods-root and stem tubers, fruit and fruit drinks, seeds and grains, greens and vegetables. Food poisoning and intoxication, prevention and cure.Chemical changes in food, food enzymes and their uses.

COURSE REQUIREMENTS:

This is a compulsory course for all 300 level Biochemistry students in the university. who are expected to have passed BCH 201 and BCH 202. Students are expected to participate in all course activities and have minimum of 75% attendance to be able to write the final examination

READING LIST:

1. Introduction to the biochemistryof foods. Z. Berk *TX 531. B69, 1976.*

2. Nelson D.L. and Cox M. M. Lehninger Principles of Biochemistry. 4th ed. 2004

3. www.whfreeman.com/lehninger

4. Garrett, R. H. and Grisham, C. M. Biochemistry 3rd ed. Brooks/Cole 2005

5. R.K. Murray, D.K. Granner and P.A. Mayes. Harper's Illustrated Biochemistry, 26th ed Lange Medical Books/McGraw-Hill 2003

6.Source- processing and preservation of tropical and subtropical foods. J. Mand Kordylas 8, TX, 599. K6

LECTURE NOTES

FOOD BIOCHEMISTRY- INTRODUCTION

The world in which we live in is composed of two main classes of matter. The inanimate and the organised living matter.

The inanimate which comprises rocks, stones and minerals is made up of such patterns as atoms, molecules and crystals. They do not change appreciably except due to erosion, weathering or cataclysm.

Living matter which comprises micro organisms, plants, animals, and human beings are also built of atoms, molecules and crystals, but are composed of extremely complex highly organised patterns which have the ability to grow, to support themselves and to reproduce. In addition, living matter is in a state of constant and rapid metabolic change

Living matter is divided into *two* general groups: the ***AUTOTROPHIC*** and the ***HETEROTROPHIC*** *organism*

The autotrophic- are capable of growth and reproduction by preparing their required complex materials from very simple inorganic compounds namely: CO_2, H_2O and few minerals utilising sunlight to provide the energy for the synthesis e.g. all green plants and a limited number of micro organisms.

The heterotrophic- are those that cannot subsist, grow or reproduce without food but have to acquire food from the autotrophic organism or by devouring other members of their groups e.g. all animals and most of the micro organisms

As a heterotroph, man seeks to obtain his food from plants and animals. At a very early stage, man was confronted with the problem of how to preserve his food from deterioration as well as from deleterious changes in colour, taste, flavour, texture and in nutritional value. Man learned the art of food preservation by trial and error, and intuition.

Scientific study of food is a comparatively new field; its beginning can be attributed to *Louis Pasteur*.

The reason for the late development and slow progress of food science may be attributed to:

1. Complexity of foods

Most foods are extremely complex mixtures of many thousands of chemical species. Three (3) groups; Carbohydrates, lipids and protein together with water constitute the bulk of most foods and usually account for more than 99% of their mass. The rest 1% is made up of hundreds of thousands of various compounds, some present only in very small quantities. Usually, the characteristics taste, odour, and colour of foods are due to those secondary components. The vitamins important for their nutritional functions are also present. The trace components may also possess undesirable physiological properties, such as inhibitory and toxic effects.

2. **Chemical activity**

Most food constituents possess chemically active groups in their molecules. They are capable of entering into complicated series of reactions with each other and with the media surrounding the food, air, water, packaging materials, and processing equipment. Many of these reactions are accelerated by the presence of the enzymes. During preparation and processing, foods are exposed to conditions of heat, moisture and concentration which induce additional reactions. Therefore, foods should be regarded as rapidly changing chemical systems. These changes may be desirable or objectionable. Understanding foods as biochemical systems is therefore of utmost importance to food technologists

3. **Physical structure**

The physical structure of foods determines an important factor of acceptability, namely the *texture*. With very few exceptions, foods are not well-defined physical systems like ideal solutions or crystalline solids. On the contrary, they are characterised by the presence of macromolecular components and phases in colloidal dispersions.

Food biochemistry is the study of the composition of foods and the reactions which leads to changes in their constitution and characteristics.

WHAT IS FOOD?

Food is composed of many chemical substances. They are responsible for the structure, colour, flavour, and nutritional value of food. Most of these chemical substances occur naturally, others are added by manufacturers. The chemical substances that give colour and flavour to foods are present in very small quantities which have no nutritional value. They make food look attractive and appetising, The remaining chemical substances are called nutrients because they are absorbed and used by the body, e.g, water, proteins, fats, carbohydrates, vitamins, and mineral elements. Dietary fibre though not absorbed, is also essential for healthy living.

Food is a generalised term which includes all the materials taken in through the mouth which are necessary for the activity, growth and well being of the body. We can divide these into the following categories:

1. Fuels to provide energy for movement and all the chemical processes going on in the body.
2. The raw materials for the growth of the body and the repair or replacement of tissues.
3. Substances concerned in regulating the chemical activities of the body.
4. Substances required for the health and protection of the body.

For man, these needs are met if the diet includes the following:

- Carbohydrates
- Fats and proteins
- Mineral salts
- Vitamins
- Water

CARBOHYDRATES

These provide energy when their molecules are broken down during respiration. They include starches and sugars, and occur in large quantities in cereals, bread, potato and most root vegetables. They all have molecules composed of carbon, hydrogen and oxygen. E.g. glucose sugar-$C_6H_{12}O_6$, sucrose-$C_{12}H_{22}O_{11}$, starch- $(C_6H_{10}O_5)n$ e.t.c.

Glycogen, a substance rather like starch is an important fuel reserved in man, most of it is stored in the liver.

FATS

These include oils, a term used for fats with low melting point, which are liquid at room temperature. Fats are also energy foods. Their molecules like those of carbohydrates consist of carbon, hydrogen, and oxygen atoms only. They differ from them in not having hydrogen and oxygen in the same proportions as in water.

Fats are present in pure form in butter, margarine, olive oil, milk, e.t.c. Fats is easily stored in the body under the skin. It acts as an insulating layer which helps to keep in the heat of the body.

PROTEINS

These can also be used as source of energy. Proteins are polymers built from amino acid units.

Our own body requires about 20 amino acids; at least 10 of these must be obtained from proteins in the diet, the remainder can be synthesised biologically. Proteins derived from animal sources such as meat, fish, cheese, eggs and milk are extremely good sources of these 10 essential amino acids.

Deficiency of protein leads to a condition referred to as kwashiorkor.

MINERAL SALTS

Mineral salts are essential because they provide many of these elements needed for growth, protection, and the regulation of metabolic processes. Of these, elements such as sodium, potassium, calcium, phosphorus and iron are required in appreciable quantities.

-Sodium in form of sodium chloride is necessary to maintain the right osmotic balance of body fluids e.g.blood.

-Calcium is needed for muscle contraction and the formation of strong bones and teeth.

-Phosphorus is needed for bone and teeth formation

-Iron is essential for making haemoglobin. Inadequate supply of iron leads to anaemia.

There are many other important elements needed for smooth functioning of the body organs.

VITAMINS

They are micro nutrient required in small amount and help in the regulation of body metabolic activities. They take part in intermediary metabolism

They are fat soluble vitamins e.g. vitamins A, D, E, and K. Also, there are water soluble vitamins e.g. vitamin B complex and vitamin C.

Most vitamins are synthesised by plants or from other sources while some are not, but ingested into the body.

Diseases caused by deficiency of various vitamins include; beriberi, scurvy, rickets, etc.

WATER

Water is an essential part of our diet for many reasons: digestion and absorption of food do not occur without it. Water is necessary for the transportation of materials within the body.

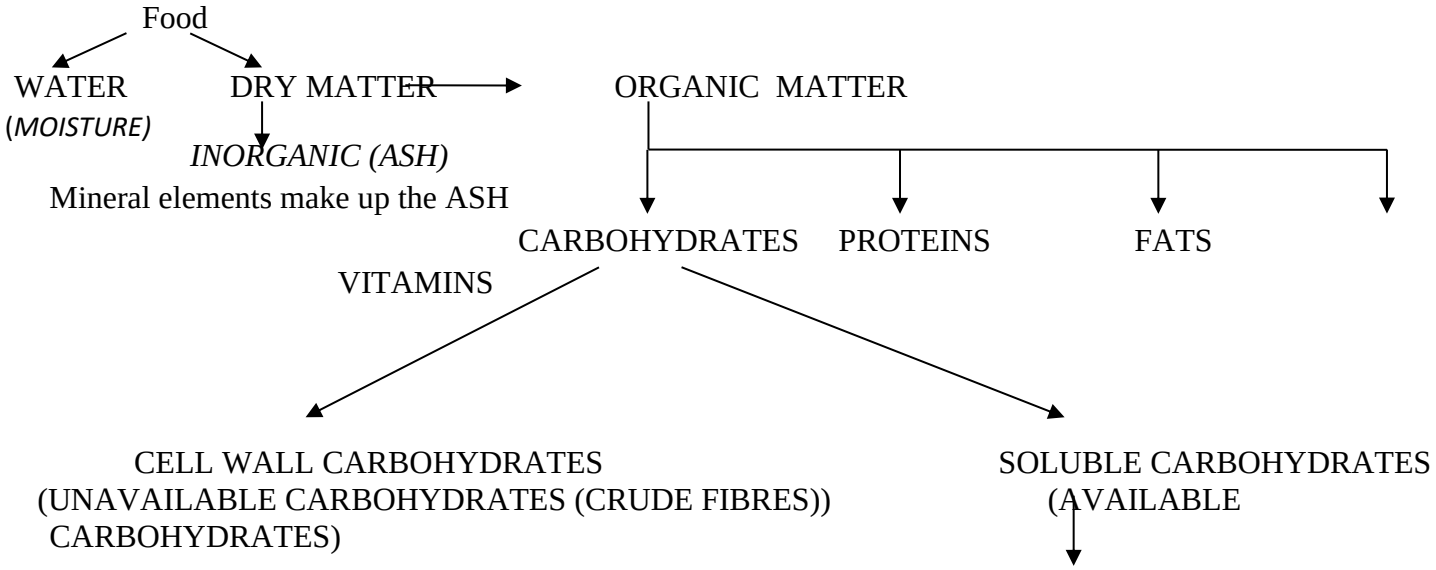
Also, all metabolic activities usually take place in watery medium.

GENERAL FUNCTIONS OF FOOD

- for energy and movement
- for growth and repair of tissues
- for regulation of health

PHYSICAL AND CHEMICAL METHODS FOR DETERMINING THE CONSTITUENTS OF FOOD

Food can be described in chemical entities and the basis for this chemical definition of food is referred to as **proximate composition**. By the proximate composition scheme, food is partitioned into six chemical entities viz: water or moisture, inorganic matter or ash, crude protein, ether extract of fats, crude fibre, and Nitrogen Free Extracts (NFE) or soluble carbohydrates. The partition is also depicted below:



The food chemist is frequently called upon to make a proximate analysis of food or food products. He may wish to ascertain the amount of the nutrients present in the food for the purpose of quality control and/or to evaluate the nutritive quality of the food. Therefore, proximate analysis of food involves the determination of the percentages of chemical entities.

Nutritive quality of food: Determination of nutritive quality of food is beyond knowing the proximate composition of the food; it also involves estimation of the digestibility and the ultimate absorption and utilization of the nutrient. Consequently, nutritive quality assessment of the food requires knowing the amount of energy derivable from the food (calorie value) and the levels of anti-nutritional factors that are present in the food.

CHEMICAL ANALYSIS OF FOOD

- *Moisture:* there are several methods available for moisture determination. They may be direct or indirect method. Some of the methods include: air oven drying, vacuum oven drying, freeze drying, chemical desiccation, thermo-gravimetric analysis, azeotropic distillation, extraction method, refractometry etc. The choice of the method used for a specific food product may depend on the following factors:

➢ Form in which water is present in the food
➢ *Nature of the food product that is, whether easily oxidised* or composed
➢ Relative amount of water present in the product
➢ Rapidity of determination
➢ Accuracy desired
➢ Availability and cost of equipment required in the determination of moisture.

i. AIR OVEN DRYING METHOD:

This method has been the most widely used for moisture determination. Modern drying ovens usually are heated electrically or by infra red heaters, and are equipped with in-built balances for rapid routine analysis of any stable solid food material. Since oven drying is based on weight-loss, the sample must be thermally stable and not contain significant quantities of volatile components.

The measurement of moisture content of sample by a conventional method employing an analytical balance with a drying oven comprises of the following operations:

❖ Tare-Weighing the drying dish
❖ Filling the dish with food material and re-weighing
❖ Drying the food material at a specific temperature until constant weight or for a specific period
❖ Cooling the dish containing the dried food sample in a dessicator
❖ Re-weighing the cool dry sample in the dish
❖ Calculating the moisture content as follows:desiccator

Let: W_1= Weight of wet sample

W_2= Weight of dry sample

W_3= W_1-W_2= Weight of moisture

$$\text{Moisture (\%)} = \frac{W_3}{W_1} \times \frac{100}{1}$$

ii. VACUUM OVEN DRYING METHOD

Some of the drawbacks of the air oven drying method can be overcome by drying the sample in a vacuum in which the sample can be heated at a lower temperature so that decomposition of the sample does not take place.

iii. FREEZE DRYING METHOD

This is the best out of the oven drying techniques as it allows the use of large samples and because the moisture is removed under milder conditions so that many other nutrients may be measured subsequently in the freeze-dried material. The method itself also produces a sample that is in many cases readily homogenized. Some residual moisture remains after freeze-drying and this must be removed under reduced pressure over a suitable desiccant. The disadvantage of this procedure is the cost of equipment and longer time at which it is achieved.

iv. CHEMICAL DESSICATION

In this method of drying, phosphorus pentoxide (PO_5) barium monoxide (BaO_2), magnesium perchlorate ($MgOCl_2$), anhydrous calcium chloride ($CaCl_2$) or concentrated sulphuric acid (H_2SO_4) are used as desiccants.

The sample is placed in a desiccator with an appropriate desiccant at room temperature. The disadvantage of this procedure is that it requires a longer time for the sample to achieve constant weight.

v. THERMO-GRAVIMETRIC ANALYSIS

The standard oven drying approach has recently been improved upon by employing semi-automatic and fully automatic thermo-gravimetric analysis methods. The most significant part of the method is a thermo-balance which measures and records automatically the weight changes of a substance while it is being heated as a function of time and temperature. Since the sample is not removed from the furnace and placed in the desiccator before weighing. The weighing error is minimized.

vi. AZEOTROPIC DISTILLATION

A type of distillation in which a substance is added to the mixture to be separated in order to form an azeotropic mixture with one or more of the components of the original mixture. The azeotrope or azeotropes then formed will have a boiling point different from the boiling points of the original mixture and will permit greater ease of separation.

Water is simultaneously distilled with another usually immiscible liquid at a constant ratio. For example, although the boiling point of water and toluene as sample components are 100^{o}C and 110^{o}C respectively, the boiling point of the binary mixture of water and toluene is 85^{o}C with the ratio of relative quantities at the boiling point being 20:80 respectively. The results of these methods have almost invariably approach the value of the theoretical water content within 1%, which apparently represent normal magnitude of error of measurement.

Azeotropic distillation method has the following merits:

- Method determines water directly and the results are actually water content values and not loss in weight
- Results are frequently more accurate than those obtained by oven drying method. This is partly due to much larger sample size with better representative sample
- Time of determination is usually relatively short from 30mins to 1hour
- Apparatus is simple and most parts are easily obtainable
- The effect of environmental humidity is eliminated
- The method prevents oxidation of the sample

vii. REFRACTOMETRY

It combines the attributes of simplicity, speed, and reasonable accuracy. In this method, the food is homogenised with anhydrous solvent such as isopropanol and equilibrated to a predetermined temperature and then the refractive index is determined. The percent (%) moisture is calculated from the data obtained from standard solutions.

viii. INDIRECT METHOD

This is physical in nature. Moisture content is not determined directly. What is measured with the aid of an instrument is a particular function that has some forms of proportional relationship with the amount of water present in the product e.g. direct current and alternating current conductivity, dielectric capacitance, sonic and ultrasonic absorption, infra red spectroscopy and NMR spectroscopy. Only the above indirect methods offer the possibility of continuous measurement and automatic control of moisture content in industrial processes.

INORGANIC MATTER DETERMINATION

The inorganic matter fraction of the food is collectively referred to as the **ash.** This inorganic matter comprises of the mineral elements, most of which are of nutritional importance to man. Consequently, the food analyst sometimes is interested in fractionating the inorganic matter of food into nutritive and non-nutritive components in acid typified of the acid secretions of the human digestive tract. Then, the food ash is fractionated into acid-soluble and acid-insoluble components.

Determination of the inorganic matter of food may be in the form of determination of total ash, soluble ash, acid-insoluble ash, and individual mineral elements.

DETERMINATION OF TOTAL ASH

- Previously, the ignited crucible was dried in the oven, cooled in a desiccator, and then tare-weighed
- Fill the crucible with the food sample that has been previously dried in the oven and weighed.
- The sample and the crucible are then transferred into the muffle furnace for ashing at a temperature between 300 and 600^{o}C for about 1-3 hours.
- The crucible with the ash is cooled in the desiccator and then weighed.
- Percentage ash is then calculated thus:

  W_1= weight of dried sample

  W_2=weight of total ash

$$\text{Total ash (\%)} = \frac{W_2}{W_1} \times \frac{100}{1}$$

FRACTIONATION OF TOTAL ASH INTO SOLUBLE ASH AND ACID-INSOLUBLE ASH

- Dissolve the ash obtained above (from the determination of ash) by boiling with 2N(normal) HCL
- Cool the solution and then filter
- Dry the residue in the oven; cool in the desiccator and then weigh (step 3)
- Calculate % soluble ash and acid-insoluble ash as follows:

  W_3= weight of dried residue (from step 3)

$$\text{Insoluble ash (\%)} = \frac{W_3}{W_1} \times \frac{100}{1}$$

Weight of soluble ash= W_2-W_3 =W_4

$$\text{Soluble ash (\%)} = \frac{W_2-W_3}{W_1} \times \frac{100}{1} \quad \text{or} \quad \frac{W4}{W_1} \times \frac{100}{1}$$

INDIVIDUAL MINERAL ELEMENTS

In most cases, instrumental methods of analysis are considered preferably to the classical gravimetric volumetric procedures, and in general offer greater specificity and sensitivity than the colorimetric method. Whenever instrumental procedures are used, it is essential to ensure that interference from other constituents has been eliminated or controlled.

This may be done by the use of standard solutions containing the interfering substances in a similar concentration to those found in the samples being examined, or by the addition of substances, which suppress the interference. This latter procedure is often of value in the atomic absorption spectrophotometry.

PREPARATION OF SAMPLE FOR MINERAL ANALYSIS

The first stage in the analysis is the destruction of the organic matter in the food. This can be done either by dry ashing (i.e. ignition in a muffle furnace in a silica or platinum crucible or by oxidation with hot concentrated acid usually mixtures of sulphuric acid, nitric acid or perchloric acid (i.e. wet oxidation). Some elements react with the material of the crucible or are converted into insoluble compounds. If the alkali metals are to be determined, it is important to ensure that the temperature in the muffle furnace does not exceed 475oc, because at high temperature, losses of these elements may occur by volatilisation.

With wet oxidation on the other hand, formation of insoluble complex may result. The ash resulting from the dry ashing is conveniently dissolved in hydrochloric acid and measurements made on the ash extract. The concentrated acid digestion mixture from the wet oxidation process is diluted before analysis.

Mineral analysis: the various methods used for the analysis of the mineral elements and their limitations are given.

Constituents	Available method	Limiting factors
Sodium Potassium	Flame photometry, or atomic absorption, spectrophotomery	Interference from other constituents must be controlled
Calcium	Atomic absorption spectrophotometry. Flame-photometry Precipitation as oxalate	Interference effects must be controlled by addition of suitable reagents
	Complex volumetric titration	Requires careful control of titration
Magnesium Iron	Atomic absorption spectrophotometry	Interferences effects must be controlled
Copper zinc manganese	Specific colorimetric procedures	Specificity of methods: usually time-consuming
Molybdenum Chromium Vanadium Selenium	Emission spectrography	Very sensitive and rapid but often associated with low precision
Phosphorus	Colorimetrically	All phosphorus must be in the orthophosphate form
Iodine	Microdistillation and titration	
Fluorine	Microdistillation followed by colorimetry	
Chloride	Potentiometric and precipitation titration	

PROTEIN DETERMINATION

This represents the nitrogenous fraction of the food. Since many proteins contain about the same amount of nitrogen which is 16%, it has become the usual practice in many laboratories to determine the % total nitrogen in food and then multiply it by a factor e.g 6.25. This general factor of 6.25 is used for most protein. Although there are exceptions to these rules. For e.g, the factor of 6.38 is used for milk protein.

The term crude protein is used since the method determines the total organic nitrogen. There are small amount of nitrogenous compounds present in the food which are not protein i.e non-protein nitrogen (NPN). NPN consist of urea nitrogen, ammonia nitrogen, uric acid
nitrogen,
amines etc. Overall, protein therefore maybe divided into two portion which are true protein and non-protein nitrogen.

Crude protein is estimated by kjeldahl digestion procedure, where heating in concentrated sulphuric acid digest the organic matter of the food. From the Nitrogen-containing organic molecules, ammonium sulphate is formed. The amount of ammonia is extimated by distillation and then titrated against a standard acid solution. % crude protein is then estimated by multiplying total % Nitrogen by 6.25

KJELDAHL NITROGEN DETERMINATION

The determination of Nitrogen by the kjeldahl method involves the following stages :

A <u>Digestion</u>

The food is heated with conc sulphuric acid in the presense of potassium sulphate and catalyst such as copper, mercury or selenium. These process brings about the conversion of the organic matter Nitrogen to ammonim hydrogen sulphate. In addition, in the process, organic matter Carbon & Hydrogen are converted to water and CO_2

Organic matter + conc H_2SO_4--------> NH_4HSO_4 + CO2 + H_2O

<u>Hints</u>

- K_2SO_4 raises the boiling point of the H_2SO_4 so that the digestion can go on for a reasonable length of time without loss of the acid by volatilisation.
- Cu, Hg or Se is used as catalyst in the reaction.
- The turning of the digest to colorless, signals the end of digestion
- Special long neck round bottom flask i.e kjeldahl flask is used for the digestion. The long neck of the flask prevent loss of the acid through volatilisation.

B <u>Distillation</u>

The clear digest is then transferred into a distillation apparatus where conc alkaline such as potassium hydroxide or NaoH is allowed to react with the ammonium hydrogen sulphate. These reaction causes the realease of ammonia gas (the distillate).

2OH- + NH_4HSO_4------> NH_3(g) + 2H_2O +SO_4

The distillate is collected in a receiver that contains standard saturated boric acid or mineral acid such as HCl and sulphuric acid for neutralisation of the ammonia and prevention of loss by volatilisation.

If HCl is used neutralisation is

H^+(excess Hcl) + NH_3--------> NH_4^+

C <u>Titration</u>

Next, the excess HCl is titrated with NaOH. The amount of NH_3(and hence the amount of nitrogen in the sample) is computed from the difference in millimoles between HCl in the receiver and NaOH needed for back-titrating the excess HCl.

If boric acid is used neutralisation reaction is

$NH_3 + H_3BO_3$----------> $NH_4^+ + H_2BO_3^-$

Borate is the conjugate base of boric acid. The borate is titrated with standard HCl or H_2SO_4 without interference from the excess of boric acid.

H^+(HCl) + $H_2BO_3^-$ ---------> H_3BO_3

TRUE PROTEIN NITROGEN DETERMINATION

These is measured after extraction and precipitation of the protein and removal of non-protein nitrogen. A large number of different precipitants have been used: all of them are to some extent empirical and need to be treated with the type of food under examination. The isolated protein is then digested with conc H_2SO_4 and the ammonia nitrogen estimated as described earlier.

AMINO ACID COMPOSITION

If the amino acid composition is desired, the protein is first hydrolysed using acid, alkali or enzyme and then the resulting amino acid are then estimated using automatic instrumentation in the form of amino acid analyser. Another widely used method of estimating amino acid is that based on ion-exchange chromatography. The limiting factor in these procedures is the hydrolytic stage particularly in the presence of Carbohydrates. Certain amino acid are lost when the protein is hydrolysed with either acid or alkali e.g tryptophan is lost incase acid is used. The application of gas-liquid chromatography techniques in the determination of amino acids is limited at present by the preparation of suitable volatile derivative.

FAT DETERMINATION

Several methods are available for determination of total fats that is dry extraction and wet extraction method.

DRY EXTRACTION METHOD (SOXHLET TYPE)

The material to be analysed must be dried thoroughly in an oven and the solvent used for the extraction must be anhydrous. This is necessary to avoid the presence of any water so that the water soluble materials are not extracted and determined with the fat. Frequently, the fat determination is made to follow moisture determination.

The procedure is based on continuous extraction of dried food with liquid solvent which have a wide spread use. However, they are known to give incomplete extraction for many foods and subject the extracted lipid to a prolonged period at high temperature. This method is time consuming, this is usually used for solid samples and result may not be accurate. It determines free fats, and fat may be destroyed on long eating.

WET EXTRACTION METHOD

The dry extraction method determines free fat and tends to exclude any bound fat unless a mixture e.g chloroform and methanol is used. To estimate the total fats, initial treatment with acids or alkali is necessary to render the bound fat free before extraction.

WERNER SCHMIDT PROCESS (Acid extraction)

In acid method, the material is heated with HCl, to destroy the protein, and the fat separate as a layer on top of the acid layer. The protein dissolves in the acid and the separated fats can be extracted by shaking at least three times with diethyl ether and light petroleum. Treatment of the original material with ammonia prior to the addition of acid is advisable with foods such as dried milk and processed cheese. The acid extraction process is less suitable than the alkaline method, if the material contains high proportion of sugar.

ROSE- GOTTLIEB PROCESS (Alkaline extraction)

For the extraction using alkali, the material is treated with ammonia and alcohol that will precipitate the protein, which dissolves in ammonia. Subsequently, the fats are extracted with ether-light petroleum mixture. Light petroleum is then added as it reduces the proportion of water in the extracts and hence, non fat soluble substance such as lactose. The alkaline extraction method gives a very accurate result provided a provided a prescribed technique is adhered to precisely. It is suitable for materials which contain much sugar.

GEBER VOLUMETRIC METHOD (for non-dry products)

The measured sample is placed in a special flask having a calibrated neck. The sample is treated with conc. Sulphuric acid which destroys the film surrounding the fat globulin permitting it to coagulate. After centrifuging, to affect a more complete separation of the fat from the aqueous phase, water is added forcing the fat column up into the calibrated neck of the flask so that the volume of the fat may be read and from these % of fat is calculated. This method is usually used for milk analysis. For instance, for certain foods, particularly non-dry products, cleaner separation can be obtained using an acetic acid-perchloric acid mixture instead of sulphuric acid.

FOLCH METHOD

This method is good for liquid and soft, semi-solid foods. The procedure of Folch, Lee and Stanley known as Folch method is by far the most frequently quoted method for fat extraction. It has not proved suitable in all circumstances but generally satisfactory modification has been given. To extract the total lipids of a wet tissue according the procedure of Folch et al (1957). You often can remove non- lipid materials present in the chloroform: methanol extract.

CARBOHYDRATE DETERMINATION

In structural terms, Carbohydrates are classified under two broad categories viz soluble and structural or cell wall Carbohydrates. Soluble Carbohydrates are present within the cellular content as simple sugars and as storage Carbohydrates i.e. starch whereas the cell wall Carbohydrates are the structural Carbohydrates.

By nutritional considerations, carbohydrates are classified as available and unavailable Carbohydrates. Available Carbohydrates are those, which are digested by the secretion of the human digestive tract.

These categories of Carbohydrates are simple sugar and starch. Unavailable Carbohydrates are those cell wall Carbohydrates i.e. cellulose, hemicelluloses, and liguin that are not dissolved by endogenous secretions of the human digestive tract.

The latter group is collectively referred to as crude fibre. In proximate analysis scheme, available Carbohydrates are collectively referred to as nitrogen free extract (NFE).

Most procedures involve the separation of free sugars from the polysaccharides by extraction with aqueous alcohol followed by the measurement either colorimetrically or enzymatically or after dilute acid hydrolysis, using glucose specific enzymatic method.

In some foods, particularly cereals, extraction with perchloric acid followed by colorimetric measurement gives reliable results. Where a value is given as a disaccharide, it could be multiplied by 1.05 and as starch 1.1

Individual sugars may be measured either using specific enzymatic technique or colometrically after separation by ion-exchange chromatography. Gas liquid chromatography is also a useful technique for separation and measurement of individual sugars. Highlights of method available for the determination of Carbohydrates are given in the table:

Methods for determination of Carbohydrates

Constituent Carbohydrates	Available method	Limiting factor
Available Carbohydrates	1. Extraction of free sugar and measurement by colorimetric procedure 2. Hydrolysis of starch enzymatically and subsequent measurement of glucose colorimrtrically 3. Extraction of starch n with perchloric acid followed by colorimetric measurement	Specificity of chemical procedure. Specificity of enzyme preparation used . Interference from other constituent
Constituent sugars	1. Ion-exchange chromatography 2. Specific enzyme procedure 3. Gas-liquid chromatography	Cost of the instrument. Purity of enzyme preparation Preparation of volatile derivatives

Unavailable Carbohydrates with other constituent of the cell wall	1. Fractional extraction and hydrolysis 2. Modification of the classical fibre procedure based on normal acid digestion.	All procedures at present are empirical to a greater or lesser extent. The procedure estimates the Carbohydrates not digested by man.

In the proximate analysis scheme, the object of carbohydrate determination is to fractionate carbohydrate into the crude fibre and NFE.

CRUDE FIBRE DETERMINATION

This represent the insoluble fraction of the food after boiling with dilute acid and alkali.

In monogastric animal it represent majority of indigestible portion of the food while in ruminant it represent the major source of energy because they have ability to digest the fibre.

The most common method of crude fibre determination is that which involves:

a) Boiling of a known amount of dried sample of the food with a mixture of trichloroacetic acid/acetic acid/nitric acid.
b) Filtering and drying the insoluble residue
c) Transferring the dried residue into the furnace for ashing and weighing the ash
d) Weight of the crude fibre is taken as the difference between the weight of dried insoluble residue and ash

NITROGEN FREE EXTRACT DETERMINATION

There is no chemical determination for NFE as a single entity. Rather it is calculated by adding together the percentages of crude protein(CP), ether extract(EE), crude fibre(CF) and ash on dry matter basis. That is % NFE=100-[Y], where [Y]=%CP + %EE + %CF + % Ash.

NFE is composed almost entirely of soluble carbohydrate. Although small amount of certain organic acids are included, but for fractional feeding purposes, NFE can be considered being synonymous to carbohydrate

NUTRITIVE QUALITY OF FOODS

Determination of nutritive quality of food is beyond knowing the proximate composition of the food. It also involves estimation of the digestibility and the ultimate absorption and utilisation of the nutrients.

Consequently, nutritive assessments of the food require knowing the amount of energy derivable from the food (i.e caloric value) protein quality and the levels of anti-nutritional factors or particles that are present in the food.

TOTAL ENERGY (CALORIC VALUE) DETERMINATION

Total energy of the food is the term used to describe the gross energy (GE) of the food. This is usually determined instrumentally using the **BOMB CALORIMETER**, **and it involves determination of the heat energy produced by 1g of the food sample when ignited in the calorimeter.**

However, for the nutritional purpose, not all the GE of the food is utilised because not all the nutrient in the food will generate energy in the biological system. The portion of the food that will be utilised for energy production in the living organism comprises of carbohydrates, proteins, and fats.

Consequently, amount of energy produced by each of the three major energy producing nutrients have been determined, and the values have been used in the theoretical estimation of food energy.

FOOD PROCESSING AND PRESERVATION

1. Food processing refers to any changes made in plant, animal or any material used for food carried out in the home or in the commercial establishment. Therefore, food processing includes food preparation, which makes food ready for immediate consumption.
Food preservation keeps the food for future consumption

Why is food processed?

- To improve nutritive value and availability to the body
- To improve its digestibility
- To improve its sanitary quality
- To create desirable flavours which are pleasant to taste
- To preserve food so saving time and energy

Food processing may cause the loss of certain desirable quality of food.

Technique of processing and preparation of food

1. **Separation and subdivision**- this is done to increase palatability or eating quality. Separation is achieved by removing the less desirable parts of the food which are distasteful, coarse in texture and unattractive in appearance. E.g. peeling of plantain to remove the tough and unappetizing skin.
Subdivision is necessary to remove portions which would otherwise cause deterioration through spoilage, e.g. degutting of fish
Method of separation and subdivision are: cutting, grinding, pounding, grating, peeling (scalding), paring (scraping), cracking, milling, filtering, floatation, pressing, refixing, skimming, steeping, evaporation and centrifugation.

2. **Combination or mixing**.
This is used to cause food material or food ingredients to be mixed, or to be combined during food preparation to create palatability of end product and control texture and flavour. Commonly used methods to achieve mixing and combining of ingredients are: beating (whipping), blending, stirring, stir-pressing, kneading, cutting, folding, creaming, marinating emulsification.

3. Heating

All substances either gain from or lose heat to the immediate environment. Heat is used in food preparation for cooking or toasting.

Cooking: is when heating affects the entire mass of the food being heated at the same time. E.g. cooking soup, boiling yam.

Toasting: is when the heat primarily affects the surface of the food substance e.g. when plantain is toasted or roasted on a direct heat, complex changes in state occurs when foods are processed by heating.

These changes depend on:

- Method of heat transfer (air, water, steam, sand, fat, combination of two or more)
- Period of heating
- Temperature reached at the surface and in the centre of the food
- Type of food being heated

Effects of cooking

- Increase digestibility
- Softens food
- Destroys disease carrying organisms
- If cooking is done in water, valuable nutrients, salts and vitamins are lost, especially if the water is thrown away.

4. Cooling

The removal of heat from food substance by cooling the environment in which they are placed. Certain foods taste better when eaten cold. Food is placed in a refrigerator or a well ice box or chest.

5. Use of chemical compounds

Certain chemical compounds are added to foods as a means of producing certain desirable chemical changes in food e.g. baking powder added to cereal flour during food preparation produces CO2. This causes the mixture to rise. Vinegar or acidic foods like tomatoes or lemon juice are added to meat to soften it. Salt is added to food to give desirable taste.

In commercial preparation of food, chemicals are added to produce desirable characteristics in palatability, flavour, and texture to appeal to consumer demand.

6. Use of micro organisms

Micro organisms which are present in food preparation may be present naturally in a food or may be added as particular ingredients e.g. fermentation of cassava mash in fufu processing.

Techniques of food preservation

Food spoilage is caused by:

a) Internal factors inherent or present within the food itself

b) And factors present in the external environment.

- **Internal factors**

These are enzymes which are contained in all plants and animal materials and are continually causing changes in their composition. Enzyme activity may be desirable as in ripening of fruits, but their continued action causes rotting which makes fruits inedible. Enzymes also help in breaking down complex plants and animal compounds to release simple elements which are used again to nourish living things.

- **The external environment**

Living organisms, temperature conditions, moisture conditions, light and oxygen.
Living organisms: bacteria, yeast, moulds, insects and rodents. Some micro organisms produce toxic substances in foods without producing noticeable changes in the eating quality. Insects and rodents cause wastages and contaminate food with hairs and faeces.

Food preservation techniques are aimed at eliminating or restricting these factors that cause or promote food spoilage.

- Controlling enzyme activity

Heating the food to temperature destroys enzymes. Cooling or freezing slow down enzyme activity. Air and light may be shut off from foods and chemicals may be added to inhibit enzyme activity with foods.

- Micro organisms are killed by heating

Sterilised foods are also enclosed in tightly sealed containers to shut off air, moisture and light (canning). Refrigeration or freezing and storage in the frozen state slow down the activities and multiplication of micro organisms.
Dehydration causes water contents of foods to be reduced to discourage the growth and activities of micro organism. Addition of chemical preservatives, salting, smoking, addition of sugar also controls micro organisms.

- Moisture is controlled by appropriate packaging. Drying agents are also added during package.
- Light is controlled by storage of food in light proof compartments, and packaging materials through which light cannot penetrate.
- Oxygen is controlled by heating to drive off air and then sealed under vacuum. Oxygen may be replaced by other gases that do not support combustion e.g. N2 and CO2. The techniques involved in these preservative methods are complicated, they are ysually employed by industrial food processing.

DETERMINATION OF CONSTITUENTS OF FOOD

Purpose of proximate analysis:

Estimation and determination of how much of the major food components, which are Moisture, CHO, Lipids, Proteins, Ash, Crude Fibre, exist in a given food. The proximate analyses therefore are:

1. Moisture Analyses

2. Crude Fat Analyses

3. Crude Protein - (Non-protein nitrogen also included) most proteins contain 16% nitrogen.

Therefore the general “protein factor” is 100/16=6.25. If we multiply the percent nitrogen by 6.25, we obtain crude protein.

4. Ash - residue after burning all organic material. Some minerals become volatile at high temperatures of burning and therefore can be lost. Also some minerals occur in the form of salts of organic acids like citrates which contain carbon and are lost.

5. CHO and Crude fiber

Total carbohydrate = 100 - [moisture + crude fat + crude protein + ash].

Crude fiber: residue left after alkaline and acid digestion of organic matter. If we subtract the total of 1-5 from 100, we get the nitrogen free extract by difference. Doing this normally underestimates nitrogen-free extract.

(1).MOISTURE ANALYSIS

The moisture content of foods varies greatly; water is a major constituent of most food products. The approximate, expected moisture content of a food can affect the choice of the method of measurement. It can also guide the analyst in determining the practical level of accuracy required when measuring moisture content, relative to other food constituents.

Importance of Water

1. Material balance

2. Meeting the standards of product

3. Product stability (prevent deterioration, mold, bacteria, insect damage)

4. Express the composition on Dry Weight Basis

5. Economic importance (H_2O is cheap)

Water Determination Methods

1. Drying methods

2. Distillation method

3. Chemical methods

4. Physical methods