3. CHAPTER III

3.1. MOISTURE CONTENT

3.1.1. Introduction

Water is an essential constituent of many foods. It may occur as an intracellular and/or extracellular component in vegetable and animal product, as a dispersing medium or solvent in a variety of products. Typical water contents of some selected foods are given in Table 3.1.

Table 3.1. Typical water contents of some selected foods (Fox and Cameron, 1989)

Products	Water (%)
Tomato	95
Lettuce	95
Cabbage	92
Beer	90
Orange	87
Apple juice	87
Milk	87
Potato	78
Banana	75
Chicken	70
Salmon, canned	67
Meat	65
Cheese	37
Bread, white	35
Jam	28
Honey	20
Butter and margarine	16
Wheat Flour	12
Rice	12
Coffee beans, roasted	5
Milk powder	4
Shortening	0

Because of the importance of water as a food constituent, an understanding of its properties and behaviour is necessary: The presence of water influences the chemical and microbiological deterioration of foods. Also, removal (drying) or freezing of water is essential to some methods of food preservation. Fundamental changes in the products may take place in both instances.

It is both necessary and important to investigate the moisture content of

foods for many reasons- the main ones being ;

1- Water is the main ingredient of many foods and water content must be known for nutritional and quality control reasons.
2- Dilution of substances such as milk, beer, cream, and butter has long been a common adulteration, which is illegal and must tested for.
3- In processed and frozen foods, water can be picked up at different stages and this must be controlled.
4- The quality of dried foods often depends on the levels of moisture left in them (e.g. milk powder), so their moisture content must be determined.

However, the accurate determination of water in foodstuffs is very difficult. The underlying difficulty arise from water occurring foods in three forms ;

a- As bound water- this is chemically bonded as water crystallisation or as hydrates.
b- As adsorbed water- this is physically bound as monolayer the surface of the food constituents.
c- As bulk or free water- this is essentially a separate constituent easily lost by evaporation or drying

Foodstuffs, due to their homogeneous nature, may contain varying amounts of each of the three types.

Numerous methods of moisture determination are available, however their involvement with the three types of water vary and as a result poor correlation between the results of the different methods is encountered.

However, the majority of the methods give reproducible results if the empirical instructions are followed closely.

3.1.2. Determination of Moisture Content

Methods of moisture determination include drying , distillation, chemical, and instrumental methods.

3.1.2.1. Drying Methods

These are based on the measurement of weight lost due to the evaporation of water at, or near, the boiling point.

Such methods are often used. However, it must be noted that the values

obtained are not necessarily a true measure of the water content. Volatile oils may be lost, and some water (bound and absorbed) is difficult to remove- this water appears to be associated with proteins present in foodstuffs.

Different temperatures of drying may result in a different amount of free water loss, so it is important to compare results obtained using the same condition.

Some foods are subjected to the decomposition with heat, such as foods with a large proportion of sugars. In such cases low drying temperature with application of a vacuum often prevent the decomposition.

The water loss on drying is subjected to many factors apart from temperature of drying, such as particle size and weight of sample used; type of dish; and position in the oven.

Rapid approximate determinations can be carried out using elevated temperatures and instruments such as the inframatic moisture meter.

3.1.2.2. Distillation Methods

The food is mixed with immiscible solvent which has a higher boiling point and a lower specific gravity than water and is then distilled.

The distilled water and solvent fall into a graduated receiver, where the water falls below the solvent and can be thus measured. It is not unusual to obtain low results, but it has advantages of needing little attention and do not separate with the water and so are not measured.

3.1.2.3. Chemical Methods

The most commonly encountered is the Karl Fischer titration. It is based on the non-stoichiometric reaction of water with iodine and sulphur dioxide in a pyridine-methanol solution. It is usual to determine the end point electrometrically. Standardisation of the reagent is carried out against a standard water in methanol solution or a pure salt hydrate.

3.1.2.4. Instrumental Methods

These include NMR (Nuclear Magnetic Resonance), NIRR (Near Infrared Reflectance), Microwaves, GC (Gas Chromatography), Refractometry, Hydrometry

and Thermal gravimetric analysis.

3.2. CARBOHYDRATES

3.2.1. Introduction

Carbohydrates constitute one of the three main classes of nutrients. They occur in food as sugars and starches, which are a major source of energy in the diet, and as cellulose which is the main component of dietary fibre. The carbohydrate content of some foods is shown in Table 3.2.

Table 3.2. Carbohydrate content of some foods (Coultate, 1993).

Food	Carbohydrate (%)
Rice (raw)	86
Cornflakes	85
Honey	76
Spaghetti (raw)	74
Weetabix	70
Jam	69
Porridge oats	66
Milk Chocolate	59
Potato drips	50
Bread (white)	45
Bread (wholemeal)	38
Fried chipped potatoes	34
Dairy ice-cream	20
Bananas	19
Boiled Potatoes	18
Grapes	16
Peanut butter	13
Apples	12

Sugars are produced in plants from carbon dioxide and water. At the same time oxygen is evolved as shown in the equation for the formation of the simple carbohydrate glucose:

$$6CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Glucose

The building-up of carbohydrate molecules by plants is accomplished by photosynthesis. Energy is required to transform the carbon dioxide and water into

carbohydrates and this is supplied by the action of sunlight on the leaves. Consequently, photosynthesis does not take place in the dark place. Animals are unable to synthesise

It is sometimes necessary to determine the particular carbohydrates present in a food material prior to making a quantitative analysis. The kind of food will, in general give some indication of the various carbohydrates to anticipate. For example one would expect to find fructose and sucrose in uncombined forms in fruit juices and honey; milk products would contain lactose and if sweetened, also sucrose; sweet potatoes would contain starch, sucrose, and glucose. Specific carbohydrates may be detected by qualitative tests which depend mainly on differences on chemical structure. However, when employing these specific tests, it is frequently necessary to separate the particular carbohydrate from other carbohydrates in order to prevent interference with the test or arriving at erroneous conclusions due to presence of other substances of a similar nature. This is particularly true for those tests involving the reducing action of the carbonyl group of sugars. In contrast, the starch-iodine sorption test is not appreciably affected by other substances and as a consequence it is a useful test for starch in the presence of large amounts of other substances.

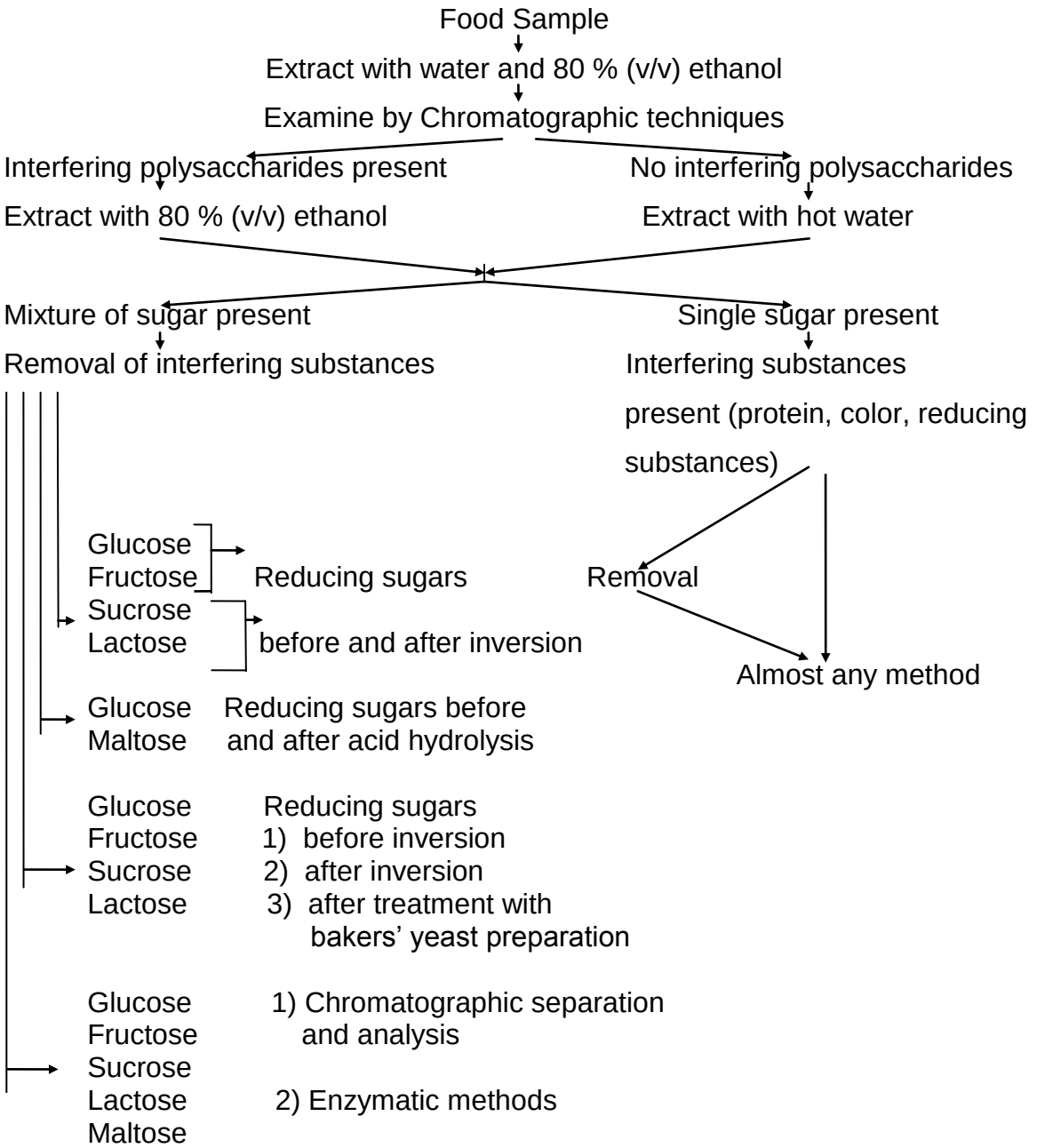
3.2.2. Determination of Carbohydrates

For many purposes a value for total free sugars, expressed possibly in terms of a monosaccharide, may be sufficient, in other cases a detailed analysis of the carbohydrate species may be required.

If only one species of sugar is present, then the choice of procedures is to a great extent much simplified, and a relatively non-specific procedures such as reducing methods, colorimetric condensation, enzymatic methods, etc. can be used. If a mixture is present, then the approach will depend on the need for values for total free sugars or whether separation or some other method is appropriate for the analysis of the mixture.

A solution of sugars can be obtained by extracting fresh or dried material with 95 % ethanol. The extract is then concentrated by rotary evaporation, filtered, and used directly for qualitative or quantitative analysis. Separation of sugars can be accomplished by chromatography on either paper or an appropriate thin layer.

Gas-liquid chromatography and high performance liquid chromatography are also available as alternative techniques. The principles involved in the choice of the methods are summarised in the following scheme.



Scheme for the choice of methods for analysis of sugars in foods.

3.3. FATS AND OILS

3.3.1. Introduction

The fatty constituents of food consists of a number of lipid substances, and

the method used for the determination of fat in foodstuff depends on the lipid present, on the other food components with which it is mixed and the occurancy and detail required from the analysis. Lipids have been defined as heterogeneous group of naturally occurring substances which are insoluble in water but soluble in organic solvents such as ether, chloroform, benzene and etc. Lipids contain carbon, hydrogen, and oxygen, while some contain phosphorus and nitrogen. The lipid in the solid form at room temperature is called fat, and in liquid form called oil. The fat content (crude or extractable fat) can be extracted by less polar solvents (diethylether). The natural fats are made up mostly of mixed triglycerides with only trace amounts of the mono and diglycerides and little or no free fatty acids. The free fatty acids are an index of the degree of hydrolysis of a triglyceride (hydrolytic rancidity); the presence of peroxides, aldehydes, and ketones are indicative of the amount of oxidative deterioration (oxidative rancidity) that has taken place in the fat.

The examination of oils for identity, purity and freshness can involve a very extensive series of physical and chemical tests. These are density, color, refractive index and slip point. The latter also describes qualitative test for identification of various oil values (those for iodine value saponification value etc.). Some rancidity tests, and examination for metals, which may promote taints or other forms of deterioration.

The total fat content consists of the additional “bound” lipids which require more polar solvents (alcohols) for their extraction. The bound lipids can be broken down by hydrolysis or other chemical treatment to yield free lipids which can then be extracted.

Thus methods for determining fat content include direct solvent extraction methods ; solubilisation extraction methods ; volumetric methods and instrumental methods.

3.3.2. Determination of Fats and Oils

3.3.2.1. Direct Solvent Extraction

The free lipid content, consisting of mainly triglycerides and free fatty acids, can be determined in foods by extracting the dried and ground (to make fat more

accessible to the solvent) material with petroleum ether (40-60°C) or diethyl ether. Such extraction are conveniently carried out in a continuous extraction apparatus of one of two types. The soxhlet type gives an intermittent extraction with excess of fresh condensed solvent in a special sample tube with a siphon attachment. The sample is held in a porous filter container such as a thimble, and extracted fat returns with the solvent when siphoned, but does not redistill with the solvent and condense back to the sample.

The Boltom or Baile-Walker type gives continuous extraction with condensed solvent dripping onto the sample contained in a thimble around which passes the hot solvent vapor. The efficiency of each method depends upon simple-pre-treatment (i.e. grinding) and choice of solvent. It has been reported that extracted material increases with polarity of the solvent from petroleum ether, diethyl ether, chloroform and acetone.

Complete extraction of neutral fat (triglycerides) is impeded by the presence of large amounts of carbohydrates, glycerol, lactic acid and other water soluble substances.

Such methods are satisfactory for establishing total fats of substances such as dry cereal-based ingredients in which the lipid fraction is mainly triglyceride.

For semi-solid and wet foods, to avoid a drying stage, the foods can be mixed calcium sulphate, anhydrous sodium sulphate or vermiculite to give a dry powdery mixture before transferring to a soxhlet thimble for extraction of the fat.

Other extractions involve use of alcohols to promote the release of protein or carbohydrate bound lipids (maximum extraction is obtained by a mixture of polar and non-polar solvents ; and hot extraction of foods with 2:1 chloroform : methanol).

3.3.2.2. Solubilisation Extraction Methods

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

The Werner-Schmid process involves adding hydrochloric acid to the sample in a boiling water bath to break down the protein, the fat separating as a layer on the top (the acid concentration is 6 M during the extraction), protein dissolves in the acid and the separated fat can be extracted with diethyl ether and petroleum ether. for

dried foods and processed cheese, the sample is first treated with ammonia. This method is not choice for materials containing a high proportion of sugar. Acid hydrolysis tends to co.-extract some non-lipid matter (to overcome the latter, the lipid in the dried , weighed and extracted state is carefully removed by petroleum ether and residual non-lipid extract dried and weighed to give by difference the total fat content).

3.3.2.3. Volumetric Methods

These involve dissolving the sample in sulphuric acid and centrifuging out the fat in specially calibrated glass vessels. Such a method is the Gerber method and it is commonly used for the routine determination of fat in Milk and dairy products. Different acids may be used for certain foods to ensure the dissolving of the foods and release of the fat.

3.3.2.4. Instrumental Methods

These include NMR (Nuclear Magnetic Resonance), NIRR (Near Infrared Reflectance), Refractometery, Specific gravity measurements, Impedance and Capacitance measurement.

3.4. PROTEINS

3.4.1. Introduction

Proteins are essential components of every living cell, and are utilised in the formation and regeneration of tissue. They contain nitrogen, carbon, hydrogen and oxygen: many contain sulphur, some contain phosphorus, and other elements such as zinc, iron and copper.

Proteins are polymers of some 21 different amino acids joined together by peptide bonds. Because of the variety of side chains that occur when these amino acids are linked together, the different proteins may have different chemical properties and widely different secondary and tertiary structures. The various amino acids are grouped on the basis of chemical nature of the side chains. The side chains may be polar or nonpolar. High levels of polar amino acids residues in a protein increase water solubility. The most polar side chains are those of the basic

and acidic amino acids. These amino acids are present at high level in the soluble albumins and globulins. In contrast, the wheat proteins, gliadin and glutenin, have low levels of polar side chains and are quite insoluble in water. The acidic amino acids may also be present in proteins in the form of their amides, glutamine and asparagine. This increases the nitrogen content of the protein. Hydroxyl groups in the side chains may become involved in ester linkages with phosphoric acid and phosphates. Sulphur amino acids may form disulphide cross-links between neighbouring peptide chains or between different parts of the same chains. Proline and hydroxyproline impose significant structural limitations on the geometry of the peptide chain.

Proteins occur in animal as well as vegetable products in important quantities. Many plant proteins are deficient in one or more of the essential amino acids. The protein content of some selected foods is listed in Table 3.3.

Table 3.3. Protein content of some selected foods (John De Man, 1990).

Foodstuff	Protein (g / 100 g)
Meat: beef	16.5
pork	10.2
Chicken (light meat)	23.4
Fish: haddock	18.3
cod	17.6
Milk	3.6
Egg	12.9
Wheat	13.3
Bread	8.7
Soybeans : dry, raw	34.1
cooked	11.0
Peas	6.3
Beans : dry, raw	22.3
cooked	7.8
Rice: white, raw	6.7
cooked	2.0
Cassava	1.6
Potato	2.0
Corn	10.0

The characteristics of proteins:

1. They are polymeric materials of high molecular weight
2. They are of colloidal dimensions and as such will not pass though semipermeable membranes
3. They are amphoteric, i.e., they may act chemically both as acids and as bases

4. Following complete hydrolysis, the hydrolysate consists entirely of amino acids
5. In their polymeric structures the amino acid units are joined together in definite sequences and in definite three dimensional conformations.

3.4.2. Determination of Protein

There are several alternative chemical and physical methods available for determination of protein content of foods.

3.4.2.1. Direct Determination of Protein

Proteins consist of amino acids which have different functional groups which show a wide variation of chemical reactions which can be used for their estimation. Foods contain mixtures of different proteins, and as a results, methods for direct determinations of protein need to be calibrated against a reference standard e.g. the Kjeldahl method.

The Kjeldahl Procedure for Nitrogen Content

The Kjeldahl method is based on the reduction of nitrogen of the nitrogenous substance into ammonia by boiling with concentrated sulphuric acid which is fixed by excess of acid as ammonium sulphate. The digest is then made by alkaline, and the liberated ammonia which is distilled off is trapped and titrated.

Simple digestion with concentrated sulphuric acid is a slow process, and many catalyst have been employed - mercuric oxide, selenium, potassium or sodium sulphate.

Traditionally, the ammonia liberated from the digest, having been made alkaline, is distilled into standard quantity of standard dilute acid which is liberated with standard alkali to give the organic nitrogen content of the sample. Nowadays, it is more usual to distil into 4 % boric acid solution and titrate with the ammonia direct with standard sulphuric acid (the exact strength and quantity of boric acid is not required).

Apparatus for macro and micro determinations are available as are automated set-ups such as Kjel-Foss, Tecator Kjeltec systems.

Conversion Factors for Nitrogen to Crude Protein

The determination of total nitrogen by Kjeldahl procedures will not include inorganic nitrogen from such nitrates and nitrites. Radiochemical methods detect nitrogen in all forms of combination. Factors are used to convert nitrogen to crude protein. These can be found in Table 3.4.

Table 3.4. Factors for the Calculation of Protein Content (Lamb, J., 1995).

Foodstuff	Factor
Wheat : wholemeal	5.83
flour, except wholemeal	5.70
macaroni	5.70
bran	6.31
Rice	5.95
Barley, oats, rye	5.83
Maize	6.25
Soya	5.71
Nuts : peanuts, brazil nuts	5.41
almonds	5.18
other nuts	5.30
Milk and milk products	6.38
Gelatine	5.55
All other foods	6.25

3.4.2.2. Formol Titration

When formalin is added to neutralised aqueous solution containing protein, the - NH_2 grouping reacts to form the methylene-imino group with the release of a proton which may be titrated.

3.4.2.3. Colorimetric Methods

The biuret reaction gives a purple coloration when the peptide bonds in a protein react with cupric ions alkaline conditions. Modifications involve the addition of propan - 2 - ol and application of heat.

Folin's reagent (phosphomolybdic - phosphotungstic acid) is reduced by proteins to form a molybdenum blue complex. This reaction has been modified and enhanced by Lowr and finds wide applications, especially in biochemical analysis.

Sulphonated acid dyes, in acidic conditions, react with proteins to form an insoluble protein - dye coagulum, which is removed by centrifugation or filtration

leaving the amount of dye left in the supernatant being indirectly protonated to the amount of protein in the sample. This method is highly empirical and needs standardisation and calibrations.

3.4.2.4. Direct Distillation

As proteins contain the amino acids asparagine and glutamine which react as amides, food proteins release ammonia when distilled with excess strong sodium hydroxide solution.

3.4.2.5. Infra-red Methods

Infra-red reflectance is used in automatic equipment for milk and grain analysis.

Near infra-red reflectance is most modern development of automatic analysis of many other solid foods.

Instrument must first be calibrated with samples of known composition.

3.5. ASH

The ash of a foodstuff is the organic residue remaining after the organic matter has been burnt away. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may be losses due to volatilisation or some interaction between constituents. Conditions of ignition are specified for various materials in standards. The ash figure can be regarded as a general measure of quality (e.g. maximum ash is prescribed by edible gelatine) and often is a useful criterion in identifying the food. When a high ash figure suggests the presence of an adulterant, it is often advisable to determine the acid insoluble ash also.

Types of Ash

a. Water Soluble Ash : The ash is boiled with 25 ml water and the liquid filtered though an ashless filter paper and thoroughly washed with hot water. The filter papers then ignited in the original dish, cooled and the water insoluble ash is weighed.

In some foods, a low water-soluble ash indicates previous abstraction of important constituents with consequent lowering of quality, e.g. ginger, tea.

b. Acid Soluble Ash : The ash is boiled with 25 ml of dilute hydrochloric acid (10 % w/w HCl) for 5 min, the liquid filtered though and ashless filter paper an thoroughly washed with hot water. The Filter paper is then ignited in the original dish, cooled and weighed. In some instance it is advisable commence by evaporating the ash to dryness with concentrated hydrochloric acid to render the silica insoluble before repeated treatment with hot dilute acid.

c. Sulphated Ash : This involves moistening the ash with concentrated sulphuric acid an igniting gently to constant weight. The sulphated ash enables a constant figure to be obtained for ashes in which varying proportions of volatile are lost according to the ignition temperature employed.

3.6. CRUDE FIBRE

Crude fibre is the organic residue which remains after the material has been treated under standardised conditions with light petroleum ether, boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution, dilute hydrochloric acid solution, alcohol, and ether.

The crude fibre consists largely of cellulose together with a little lignin. As the recovery of cellulose using the specified procedure seldom exceeds four-fifths of that actually present, the crude fibre does not represent a measure of specified group of substances. Also as the figure obtained tends to vary with the conditions employed, it is important to adopt a standardised procedure in order to obtain constituent results.