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**COURSE NAME: POSTGRADUATE DIPLOMA IN HUMAN NUTRITION**

**ASSIGNEMENT 8**

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**FOOD ANALYSIS**

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**Question One**

1. **Explain the different characteristics of foods are analyzed.**

Food analysis is the discipline dealing with development, application and study of analytical procedures for characterizing the properties of foods and their constituents. The analytical procedures are used to provide information about foods. Food analysts are interested in obtaining information about a variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes (Jiang. [B,](https://www.sciencedirect.com/science/article/pii/B9780444525123000528?via%3Dihub" \l "!)  [Tsao](https://www.sciencedirect.com/science/article/pii/B9780444525123000528?via%3Dihub#!). R, [Li](https://www.sciencedirect.com/science/article/pii/B9780444525123000528?via%3Dihub#!). Y, M, 2014).

* **Composition**

The composition of a food largely determines its safety, nutrition, physicochemical properties, quality attributes and sensory characteristics. Most foods are compositionally complex materials made up of a wide variety of different chemical constituents. Their composition can be specified in a number of different ways depending on the property that is of interest to the analyst and the type of analytical procedure used: specific atoms (*e.g.,*Carbon, Hydrogen, Oxygen, Nitrogen, Sulfur, Sodium, etc.); specific molecules (*e.g.,*water, sucrose, tristearin, b-lactoglobulin types of molecules (*e.g.,*fats, proteins, carbohydrates, fiber, minerals), or specific substances (*e.g.,*peas, flour, milk, peanuts, butter). Government regulations state that the concentration of certain food components must be stipulated on the nutritional label of most food products, and are usually reported as specific molecules (*e.g.,*vitamin A) or types of molecules (*e.g.,*proteins).

* **Structure**

The structural organization of the components within a food also plays a large role in determining the physicochemical properties, quality attributes and sensory characteristics of many foods. Hence, two foods that have the same composition can have very different quality attributes if their constituents are organized differently. For example, a carton of ice cream taken from a refrigerator has a pleasant appearance and good taste, but if it is allowed to melt and then is placed back in the refrigerator its appearance and texture change dramatically and it would not be acceptable to a consumer. Thus, there has been an adverse influence on its quality, even though its chemical composition is unchanged, because of an alteration in the structural organization of the constituents caused by the melting of ice and fat crystals. Another familiar example is the change in egg white from a transparent viscous liquid to an optically opaque gel when it is heated in boiling water for a few minutes. Again, there is no change in the chemical composition of the food, but its physiochemical properties have changed dramatically because of an alteration in the structural organization of the constituents caused by protein unfolding and gelation. The structure of a food can be examined at several different levels:

* ***Molecular structure***(~ 1- 100 nm)*.*Ultimately, the overall physicochemical properties of a food depend on the type of molecules present, their three-dimensional structure and their interactions with each other. It is therefore important for food scientists to have analytical techniques to examine the structure and interactions of individual food molecules.
* ***Microscopic structure***(~ 10 nm - 100 mm)*.*The microscopic structure of a food can be observed by microscopy (but not by the unaided eye) and consists of regions in a material where the molecules associate to form discrete phases, *e.g.,*emulsion droplets, fat crystals, protein aggregates and small air cells.
* ***Macroscopic structure***(~ > 100 mm)*.*This is the structure that can be observed by the unaided human eye, *e.g.,*sugar granules, large air cells, raisons, chocolate chips.

The forgoing discussion has highlighted several different levels of structure that are important in foods. All of these different levels of structure contribute to the overall properties of foods, such as texture, appearance, stability and taste. In order to design new foods, or to improve the properties of existing foods, it is extremely useful to understand the relationship between the structural properties of foods and their bulk properties. Analytical techniques are therefore needed to characterize these different levels of structure. A number of the most important of these techniques are considered in this course.

* **Physicochemical Properties**

The physiochemical properties of foods (rheological, optical, stability, flavor) ultimately determine their perceived quality, sensory attributes and behavior during production, storage and consumption.

* **The *optical properties***of foods are determined by the way that they interact with electromagnetic radiation in the visible region of the spectrum, *e.g.,*absorption, scattering, transmission and reflection of light. For example, full fat milk has a whiter appearance than skim milk because a greater fraction of the light incident upon the surface of full fat milk is scattered due to the presence of the fat droplets.
* **The *rheological properties***of foods are determined by the way that the shape of the food changes, or the way that the food flows, in response to some applied force. For example, margarine should be spreadable when it comes out of a refrigerator, but it must not be so soft that it collapses under its own weight when it is left on a table.
* **The *stability***of a food is a measure of its ability to resist changes in its properties over time. These changes may be chemical, physical or biological in origin. *Chemical stability*refers to the change in the type of molecules present in a food with time due to chemical or biochemical reactions, *e.g.,*fat rancidity or non-enzymatic browning. *Physical stability*refers to the change in the spatial distribution of the molecules present in a food with time due to movement of molecules from one location to another, *e.g.,*droplet creaming in milk. *Biological stability*refers to the change in the number of microorganisms present in a food with time, *e.g.,*bacterial or fungal growth.
* **The *flavor***of a food is determined by the way that certain molecules in the food interact with receptors in the mouth (taste) and nose (smell) of human beings. The perceived flavor of a food product depends on the type and concentration of flavor constituents within it, the nature of the food matrix, as well as how quickly the flavor molecules can move from the food to the sensors in the mouth and nose. Analytically, the flavor of a food is often characterized by measuring the concentration, type and release of flavor molecules within a food or in the headspace above the food.

Foods must therefore be carefully designed so that they have the required physicochemical properties over the range of environmental conditions that they will experience during processing, storage and consumption, *e.g.,*variations in temperature or mechanical stress. Consequently, analytical techniques are needed to test foods to ensure that they have the appropriate physicochemical properties.

* **Sensory Attributes**

Ultimately, the quality and desirability of a food product is determined by its interaction with the sensory organs of human beings, *e.g.,* vision, taste, smell, feel and hearing. For this reason, the sensory properties of new or improved foods are usually tested by human beings to ensure that they have acceptable and desirable properties before they are launched onto the market. Even so, individuals' perceptions of sensory attributes are often fairly subjective, being influenced by such factors as current trends, nutritional education, climate, age, health, and social, cultural and religious patterns. To minimize the effects of such factors a number of procedures have been developed to obtain statistically relevant information. For example, foods are often tested on statistically large groups of untrained consumers to determine their reaction to a new or improved product before full-scale marketing or further development. Alternatively, selected individuals may be trained so that they can reliably detect small differences in specific qualities of particular food products, *e.g.,*the mint flavor of a chewing gum.

Although sensory analysis is often the ultimate test for the acceptance or rejection of a particular food product, there are a number of disadvantages: it is time consuming and expensive to carry out, tests are not objective, it cannot be used on materials that contain poisons or toxins, and it cannot be used to provide information about the safety, composition or nutritional value of a food. For these reasons objective analytical tests, which can be performed in a laboratory using standardized equipment and procedures, are often preferred for testing food product properties that are related to specific sensory attributes. For this reason, many attempts have been made to correlate sensory attributes (such as chewiness, tenderness, or stickiness) to quantities that can be measured using objective analytical techniques, with varying degrees of success.

**b. Explain the criteria used in selecting an appropriate Technique for food analysis**.

There are usually a number of different analytical techniques available to determine a particular property of a food material. It is therefore necessary to select the most appropriate technique for the specific application. The analytical technique selected depends on the property to be measured, the type of food to be analyzed and the reason for carrying out the analysis (Nielsen. S.S, 1998), Pomeranz, Y and C.E. Meloan, C.E, (1994), Gruenwedel, D.W and Whitaker, J.R. (1984).

Some of the criteria that are important in selecting an instrumental analytical technique are listed below: accuracy, precision, sensitivity, selectivity, robustness, ruggedness, scale of operation, analysis time, availability of equipment, and cost.

* **Accuracy**

Accuracy is how closely the result of an experiment agrees with the “true” or expected result. e.*g.,* fat content, or sodium concentration We can express accuracy as an absolute error, e

e=obtainedresult−expectedresult or as a percentage relative error, %er

%er =obtainedresult−expectedresultexpectedresult×100

A method’s accuracy depends on many things, including the signal’s source, the value of kA and the ease of handling samples without loss or contamination. In general, methods relying on total analysis techniques, such as gravimetry and titrimetry, produce results of higher accuracy because we can measure mass and volume with high accuracy, and because the value of kA is known exactly through stoichiometry.

* **Precision**

Precision is a measure of the ability to reproduce a result by a specific analyst (or group of analysts) using the same equipment and experimental approach keeping other conditions unchanged. When a sample is analyzed several times, the individual results are rarely the same. Instead, the results are randomly scattered. Precision is a measure of this variability. The closer the agreement between individual analyses, the more precise the results. A method’s precision depends on several factors, including the uncertainty in measuring the signal and the ease of handling samples reproducibly. In most cases we can measure the signal for a total analysis method with a higher precision than the corresponding signal for a concentration method.

* **Sensitivity**

measure of the lowest concentration of the component that can be detected by a given procedure. The ability to demonstrate that two samples have different amounts of analyte is an essential part of many analyses. A method’s **sensitivity** is a measure of its ability to establish that such differences are significant. Sensitivity is often confused with a method’s **detection limit**, which is the smallest amount of analyte that we can determine with confidence.

**Specificity:** A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components e.g., Fructose in the presence of sucrose or glucose.An analytical method is specific if its signal depends only on the analyte. Although **specificity**is the ideal, few analytical methods are completely free from the influence of interfering species.

* **Safety**.

Many reagents and procedures used in food analysis are potentially hazardous *e.g.* strong acids or bases, toxic chemicals or flammable materials.

* **Robustness and Ruggedness**

For a method to be useful it must provide reliable results. Unfortunately, methods are subject to a variety of chemical and physical interferences that contribute uncertainty to the analysis. When a method is relatively free from chemical interferences, we can use it on many analytes in a wide variety of sample matrices. Such methods are considered **robust.**

Random variations in experimental conditions also introduces uncertainty. If a method’s **sensitivity**, k, is too dependent on experimental conditions, such as temperature, acidity, or reaction time, then a slight change in any of these conditions may give a significantly different result. A rugged method is relatively insensitive to changes in experimental conditions.

* **Simplicity of operation.**

A measure of the ease with which relatively unskilled workers may carry out the analysis.

* **Destructive/Nondestructive**

In some analytical methods the sample is destroyed during the analysis, whereas in others it remains intact.

* **On-line/Off-line**

Some analytical methods can be used to measure the properties of a food during processing, whereas others can only be used after the sample has been taken from the production line.

* **Official Approval.**

Various international bodies have given official approval to methods that have been comprehensively studied by independent analysts and shown to be acceptable to the various organizations involved, *e.g.,* ISO, AOAC, AOCS.

* **Reproducibility.**

A measure of the ability to reproduce result using the same experimental approach in same as well as different laboratories using same/different equipment.

* **Speed.**

Analysis of single sample or the number of samples in a given time

* **Equipment, Time, and Cost**

Finally, we can compare analytical methods with respect to equipment needs, the time to complete an analysis, and the cost per sample. Methods relying on instrumentation are equipment-intensive and may require significant operator training. For example, the graphite furnace atomic absorption spectroscopic method for determining lead in water requires a significant capital investment in the instrument and an experienced operator to obtain reliable results. Other methods, such as titrimetry, require less expensive equipment and less training.

The time to complete an analysis for one sample is often fairly similar from method to method. This is somewhat misleading, however, because much of this time is spent preparing solutions and gathering together equipment. Once the solutions and equipment are in place, the sampling rate may differ substantially from method to method. Additionally, some methods are more easily automated. This is a significant factor in selecting a method for a laboratory that handles a high volume of samples.

The cost of an analysis depends on many factors, including the cost of equipment and reagents, the cost of hiring analysts, and the number of samples that can be processed per hour. In general, methods relying on instruments cost more per sample then other methods.

* **Nature of food matrix.**

The composition, structure and physical properties of the matrix material surrounding the analyte often influences the type of method that can be used to carry out an analysis e.g. whether the matrix is solid or liquid, transparent or opaque, polar or nonpolar. If there are a number of alternatives methods available for measuring a certain property of a food, the choice of a particular method will depend on which of the above criteria is most important

**Question Two.**

**Discuss the changes that may occur in a sample before actual analysis and how they can be prevented.**

Sample *-* A fraction of the population selected for analysis. The sample may be comprised of one or more sub-samplesselected from different regions within the population. A sample is ‘‘a smaller (but hopefully representative) collection of units from a population used to determine truths about population’’ (Field, 2005). Once we have selected our sample we have to ensure that it does not undergo any significant changes in its properties from the moment of sampling to the time when the actual analysis is carried out. Undesirable changes include: enzymatic, chemical, microbial or physical changes.

* **Enzymatic browning**.

Enzymatic browning is one of the most important reactions that takes place in most fruits and vegetables as well as in seafood. These processes affect the taste, color, and value of such foods (**Holderbaum, Daniel, 2010).** Generally, it is a chemical reaction involving [polyphenol oxidase](https://en.wikipedia.org/wiki/Polyphenol_oxidase), [catechol oxidase](https://en.wikipedia.org/wiki/Catechol_oxidase), and other [enzymes](https://en.wikipedia.org/wiki/Enzyme) that create [melanins](https://en.wikipedia.org/wiki/Melanin) and [benzoquinone](https://en.wikipedia.org/wiki/Benzoquinone) from [natural phenols](https://en.wikipedia.org/wiki/Natural_phenol). Enzymatic browning (also called oxidation of foods) requires exposure to [oxygen](https://en.wikipedia.org/wiki/Oxygen). It begins with the oxidation of [phenols](https://en.wikipedia.org/wiki/Phenols) by [polyphenol oxidase](https://en.wikipedia.org/wiki/Polyphenol_oxidase) into [quinones](https://en.wikipedia.org/wiki/Quinone) (**Macheix, J. J.; Sapis, J. C.; Fleuriet, A. 1991).**

* **Chemical change**

A chemical change occurs as the result of a chemical reaction. During a chemical reaction, the atoms within a substance are rearranged into different combinations. For example, sugar undergoes a chemical change when it is cooked to make caramel. The heat from the cooking converts sugar molecules into different molecules that give caramel its color and flavor.

* **Microbial growth**

When microorganisms grow in food, they cause varying degrees of change in the food's characteristics because of metabolic activity. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning, are undesirable. The most important factors that affect microbial growth in foods can be summarized in the following categories: (i) “Intrinsic factors” related to the food itself which include (Nutrient content, water activity, pH value, and the presence of antimicrobial substances); (ii) “Extrinsic factors” related to the environment in which the food is stored, including (Temperature of storage, and the composition of gases and relative humidity in the atmosphere surrounding the food); (iii) “Implicit factors” related to the microorganisms themselves, including (interactions between the microorganisms contaminating the food and between these microorganisms and the food), e.g., their abilities to utilize different nutrient sources, tolerate stresses, and produce promoters or inhibitors of growth of other microorganisms, etc.; (iv) “Processing factors” include treatments such as (heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain in the food after treatment). Eventually, (v) “The combined effects” interaction between the above-described factors can also affect the growth of microorganisms in foods in a complicated way; the combined effects may be additive or synergistic (Mohamed Saad Abo-Elenain, 2017).

* **Physical change**

A physical change is any change in matter that involves the substance going from one physical state to another. No [new substance](https://www.thoughtco.com/what-is-a-pure-substance-608507) is created during a [physical change](https://www.thoughtco.com/definition-of-physical-change-605910), although the matter takes a different form. The size, shape, and color of matter may change. Physical changes occur when substances are mixed but don't chemically react. The reference to a physical state involves solids, liquids, and gases. When a substance undergoes a physical change, it does not lose its original properties when changing from one phase to another. The types of physical changes can vary. A substance can go from a solid to a liquid, a liquid to a gas, a gas to a liquid, a liquid to a solid, a solid to a gas, or a gas to a solid.

**There are several ways these changes can be prevented**.

**Enzymatic Inactivation***.*Many foods contain active enzymes they can cause changes in the properties of the food prior to analysis, *e.g.,*proteases, cellulases, lipases, etc. If the action of one of these enzymes alters the characteristics of the compound being analyzed, then it will lead to erroneous data and it should therefore be inactivated or eliminated. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control enzyme activity, with the method used depending on the type of food being analyzed and the purpose of the analysis.

**Lipid Protection***.*Unsaturated lipids may be altered by various oxidation reactions. Exposure to light, elevated temperatures, oxygen or pro-oxidants can increase the rate at which these reactions proceed. Consequently, it is usually necessary to store samples that have high unsaturated lipid contents under nitrogen or some other inert gas, in dark rooms or covered bottles and in refrigerated temperatures. Providing that they do not interfere with the analysis antioxidants may be added to retard oxidation.

**Microbial Growth and Contamination***.*Microorganisms are present naturally in many foods and if they are not controlled they can alter the composition of the sample to be analyzed. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control the growth of microbes in foods.

**Physical Changes.**A number of physical changes may occur in a sample, *e.g.,*water may be lost due to evaporation or gained due to condensation; fat or ice may melt or crystallize; structural properties may be disturbed. Physical changes can be minimized by controlling the temperature of the sample the low temperature, and the forces that it experiences.

**Question Three**

**Explain the principle of moisture determination by evaporation devices, distillations methods, chemical reaction methods and physical methods.**

Moisture content is one of the most commonly measured properties of food materials. It is important to reliably measure moisture content of a food. Analytical techniques developed for this purpose, vary in their accuracy, cost, speed, sensitivity, specificity, ease of operation, *etc*.

* **Evaporation Methods**

**Principles**

These methods rely on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation:

https://people.umass.edu/~mcclemen/Moisture1.gif

Here, *M*INITIAL and *M*DRIED are the mass of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, *e.g.,*lipids, proteins, carbohydrates and minerals. Sometimes a related parameter, known as the *total solids,*is reported as a measure of the moisture content. The total solids content is a measure of the amount of material remaining after all the water has been evaporated:

https://people.umass.edu/~mcclemen/Moisture2.gif

Thus, %Total solids = (100 - %Moisture). To obtain an accurate measurement of the moisture content or total solids of a food using evaporation methods it is necessary to remove all of the water molecules that were originally present in the food, without changing the mass of the food matrix. This is often extremely difficult to achieve in practice because the high temperatures or long times required to remove all of the water molecules would lead to changes in the mass of the food matrix, *e.g.,*due to volatilization or chemical changes of some components. For this reason, the drying conditions used in evaporation methods are usually standardized in terms of temperature and time so as to obtain results that are as accurate and reproducible as possible given the practical constraints. Using a standard method of sample preparation and analysis helps to minimize sample-to-sample variations within and between laboratories.

* **Evaporation Devices**

The thermal energy used to evaporate the water from a food sample can be provided directly (*e.g.,* transfer of heat from an oven to a food) or indirectly (*e.g.,* conversion of electromagnetic radiation incident upon a food into heat due to absorption of energy by the water molecules).

***Convection and forced draft ovens****.* Weighed samples are placed in an oven for a specified time and temperature (*e.g.* 3 hours at 100°C) and their dried mass is determined, or they are dried until they reach constant mass. Temperature variation occurs within convection ovens. *Forced draft ovens* that circulates the air achieves more uniform temperature distribution within the oven. Samples that contain significant quantities of carbohydrates that might undergo chemical changes or volatile materials other than water should not be dried in a convection or forced draft oven. Many official methods of analysis are based on forced draft ovens

***Vacuum oven****.* Weighed samples are placed under reduced pressure (typically 25-100 mm Hg) in a vacuum oven for a specified time and temperature and their dried mass is determined. The thermal energy used to evaporate the water is applied directly to the sample *via*the metallic shelf that it sits upon. There is an air inlet and outlet to carry the moisture lost from the sample out of the vacuum oven, which prevents the accumulation of moisture within the oven. The boiling point of water is reduced when it is placed under vacuum. Drying foods in a vacuum oven therefore has a number of advantages over conventional oven drying techniques. If the sample is heated at the same temperature, drying can be carried out much quicker. Alternatively, lower temperatures can be used to remove the moisture (*e.g.*70oC instead of 100 oC), and so problems associated with degradation of heat labile substances can be reduced. A number of vacuum oven methods are officially recognized.

***Microwave oven****.* Weighed samples are placed in a microwave oven for a specified time and power-level and their dried mass is weighed. Alternatively, weighed samples may be dried until they reach a constant final mass - analytical microwave ovens containing balances to continuously monitor the weight of a food during drying are commercially available. The water molecules in the food evaporate because they absorb microwave energy, which causes them to become thermally excited. The major advantage of microwave methods over other drying methods is that they are simple to use and rapid to carry out (**Mo, and Tjornhom 1978).** Nevertheless, care must be taken to standardize the drying procedure and ensure that the microwave energy is applied evenly across the sample. A number of microwave oven drying methods are officially recognized.

***Infrared lamp drying****.* The sample to be analyzed is placed under an infrared lamp and its mass is recorded as a function of time. The water molecules in the food evaporate because they absorb infrared energy, which causes them to become thermally excited. One of the major advantages of infrared drying methods is that moisture contents can be determined rapidly using inexpensive equipment, *e.g.,*10-25 minutes. This is because the IR energy penetrates into the sample, rather than having to be conducted and converted inwards from the surface of the sample. To obtain reproducible measurements it is important to control the distance between the sample and the IR lamp and the dimensions of the sample. IR drying methods are not officially recognized for moisture content determinations because it is difficult to standardize the procedure. Even so, it is widely used in industry because of its speed and ease of use.

* **Distillation Methods**

These are based on *direct* measurement of the amount of water removed from a food sample by evaporation: **%Moisture = 100 (*M*WATER/*M*INITIAL).** In contrast, evaporation methods are based on *indirect* measurement of the amount of water removed from a food sample by evaporation:

**%Moisture = 100 (*M*INITIAL - *M*DRIED)/*M*INITIAL**.

Basically, distillation methods involve heating a weighed food sample (*M*INITIAL) in the presence of an organic solvent that is immiscible with water. The water in the sample evaporates and is collected in a graduated glass tube where its mass is determined (*M*WATER). (Thiex and Van Erem, 2002)

Distillation methods are best illustrated by examining a specific example: The Dean and Stark method. A known weight of food is placed in a flask with an organic solvent such as xylene or toluene. The organic solvent must be insoluble with water; have a higher boiling point than water; be less dense than water; and be safe to use. The flask containing the sample and the organic solvent is attached to a condenser by a side arm and the mixture is heated. The water in the sample evaporates and moves up into the condenser where it is cooled and converted back into liquid water, which then trickles into the graduated tube. When no more water is collected in the graduated tube, distillation is stopped and the volume of water is read from the tube **(Thiex. N, 2002).**

* **Chemical reaction Methods**

Reactions between water and certain chemical reagents can be used as a basis for determining the concentration of moisture in foods. In these methods a chemical reagent is added to the food that reacts specifically with water to produce a measurable change in the properties of the system, *e.g.,*mass, volume, pressure, pH, color, conductivity. Measurable changes in the system are correlated to the moisture content using calibration curves (Smith. V and Patel. E, 1998). To make accurate measurements it is important that the chemical reagent reacts with all of the water molecules present, but not with any of the other components in the food matrix. Two methods that are commonly used in the food industry are the *Karl-Fisher titration*and *gas production*methods. Chemical reaction methods do not usually involve the application of heat and so they are suitable for foods that contain thermally labile substances that would change the mass of the food matrix on heating (*e.g.,*food containing high sugar concentrations) or foods that contain volatile components that might be lost by heating (*e.g.*spices and herbs).(Robertson and Windham, 1983)

**Karl-Fisher****methods**. The Karl-Fisher titration is often used for determining the moisture content of foods that have low water contents (*e.g.*dried fruits and vegetables, confectionary, coffee, oils and fats). It is based on the following reaction:

2H2O + SO2 + I2 = H2SO4 + 2HI

This reaction was originally used because HI is colorless, whereas I2 is a dark reddish-brown color, hence there is a measurable change in color when water reacts with the added chemical reagents. Sulfur dioxide and iodine are gaseous and would normally be lost from solution. For this reason, the above reaction has been modified by adding solvents (*e.g.,*C5H5N) that keep the S2O and I2 in solution, although the basic principles of the method are the same (Robertson and Windham, 1983).The food to be analyzed is placed in a beaker containing solvent and is then titrated with *Karl Fisher reagent*(a solution that contains iodine). While any water remains in the sample the iodine reacts with it and the solution remains colorless (HI), but once all the water has been used up any additional iodine is observed as a dark red brown color (I2). The volume of iodine solution required to titrate the water is measured and can be related to the moisture content using a pre-prepared calibration curve. The precision of the technique can be improved by using electrical methods to follow the end-point of the reaction, rather than observing a color change. (Thiex and Van Erem 1999**).** Relatively inexpensive commercial instruments have been developed which are based on the Karl-Fisher titration, and some of these are fully automated to make them less labor intensive.

**Gas production methods.** Commercial instruments are also available that utilize specific reactions between chemical reagents and water that lead to the production of a gas. For example, when a food sample is mixed with powdered calcium carbide the amount of acetylene gas produced is related to the moisture content.

CaC2 + 2H2O https://people.umass.edu/~mcclemen/Moisture4.gifC2H2(gas) + Ca(OH)2

The amount of gas produced can be measured in a number of different ways, including (i) the volume of gas produced, (ii) the decrease in the mass of the sample after the gas is released, and (iii) the increase in pressure of a closed vessel containing the reactants.

* **Physical Methods**

A number of analytical methods have been developed to determine the moisture content of foods that are based on the fact that water has appreciably different bulk physical characteristics than the food matrix,*e.g.*density, electrical conductivity or refractive index. These methods are usually only suitable for analysis of foods in which the composition of the food matrix does not change significantly, but the ratio of water-to-food matrix changes (Richardson 1996b). For example, the water content of oil-in-water emulsions can be determined by measuring their density or electrical conductivity because the density and electrical conductivity of water are significantly higher than those of oil. If the composition of the food matrix changes as well as the water content, then it may not be possible to accurately determine the moisture content of the food because more than one food composition may give the same value for the physical property being measured. In these cases, it may be possible to use a combination of two or more physical methods to determine the composition of the food, *e.g.,*density measurements in combination with electrical conductivity measurements (**Kellans and Church, 2002)**

**Question Four**

**Describe analysis of lipid oxidation by oxygen uptake, TBARs and Peroxide Value methods.**

Lipid oxidation analysis in food samples is a relevant topic since the compounds generated in the process are related to undesirable sensory and biological effects. Proper measurement of lipid oxidation remains a challenging task since the process is complex and depends on the type of lipid substrate, the oxidation agents and the environmental factors. A great variety of methodologies have been developed and implemented so far, for determining both primary and secondary oxidation products. Most common methods and classical procedures are described, including peroxide value, TBARS analysis and chromatography. Some other methodologies such as chemiluminescence, fluorescence emission, Raman spectroscopy, infrared spectroscopy or magnetic resonance provide interesting and promising results. Therefore, attention should be paid to these alternative techniques in the area of food lipid oxidation analysis (Barriuso B, Astiasarán I, Ansorena D. 2013).

Foods which contain high concentrations of unsaturated lipids are susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, as it leads to formation of off-flavors and potentially toxic compounds. Lipid oxidation is an extremely complex process involving numerous reactions that give rise to various chemical and physical changes in lipids:

reactants → primary products → secondary products

(unsaturated lipids and O2) → (peroxides and conjugated dienes) → (ketones, aldehydes, alcohols, hydrocarbons)

Various methods to characterize the extent of lipid oxidation in foods and to determine whether or not a particular lipid is susceptible to oxidation are outlined below.

* **Oxygen Uptake**

Lipid oxidation is a major deteriorative reaction of edible oils and lipid-containing foods, leading to the generation of rancid odors and off-flavors, nutritional losses, and the consequent decrease of shelf-life (Falowo, A. B., Fayemi, P. O., & Muchenje, V. 2014). Lipid oxidation depends on the reaction between unsaturated fatty acids and oxygen. Thus, it is possible to monitor the rate at which it occurs by measuring the uptake of oxygen by the sampleas the reaction proceeds. The lipid is placed in a sealed container and the amount of oxygen that must be input into the container to keep the oxygen concentration in the head-space above the sample constant is measured. The more oxygen that has to be fed into the container, the faster the rate of lipid oxidation. This technique measures the reduction in the concentration of reactants.

* **Thiobarbituric acid (TBA)**

This is one of the most widely used tests for determining the extent of lipid oxidation. It measures the concentration of relatively polar secondary reaction products, *i.e.,*aldehydes. The lipid to be analyzed is dissolved in a suitable non-polar solvent which is contained within a flask. An aqueous solution of TBA reagent is added to the flask and the sample is shaken, which causes the polar secondary products to be dissolved in it. After shaking the aqueous phase is separated from the non-polar solvent, placed in a test-tube, and heated for 20 minutes in boiling water, which produces a pink color. The intensity of this pink color is directly related to the concentration of TBA-reactive substances in the original sample and is determined by measuring its absorbance at 540 nm using a UV-visible spectrophotometer. The principle source of color is the formation of a complex between TBA and *malanoaldehyde,*although some other secondary reaction products can also react with the TBA reagent. For this reason, this test is now usually referred to as the thiobarbituric acid reactive substances (TBARS) method. TBARS is an example of a measurement of the increase in concentration of secondary reaction products. This test is now referred to as thiobarbituric acid reactive substances (TBARS) method. TBARS method measures increase in concentration of secondary reaction products**.**

* **Peroxide value**

Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation. One of the most commonly used methods to determine peroxide value utilizes the ability of peroxides to liberate iodine from potassium iodide**.** The lipid is dissolved in a suitable organic solvent and an excess of KI is added:

ROOH + KIexcess → ROH + KOH + I2

Once the reaction has gone to completion, the amount of ROOH that has reacted can be determined by measuring the amount of iodine formed. This is done by titration with sodium thiosulfate and a starch indicator:

I2 + starch + 2Na2S2O3 (blue) → 2NaI + starch + Na2S4O6 (colorless)

The amount of sodium thiosulfate required to titrate the reaction is related to the concentration of peroxides in the original sample.

problems associated with use of peroxide value as an indication of lipid oxidation are:

* peroxides are primary products that are broken down in the latter stages of lipid oxidation. Thus, a low value of PV may represent either the initial or final stages of oxidation.
* the results of the procedure are highly sensitive to the conditions used to carry out the experiment, and so the test must always be standardized.

This technique measures increase in concentration of primary reaction products.

**Questions Five.**

**Describe protein determination by Kjeldahl, Dumas methods and UV-visible techniques**.

Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.,*tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.,*their ability to provide desirable appearance, texture or stability. Typically, proteins are used as gelling agents, emulsifiers, foaming agents and thickeners. Many food proteins are enzymes which are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or detrimental effect on the overall properties of foods. Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods.

* **Kjeldahl methods**

The Kjeldahl method or Kjeldahl digestion in [analytical chemistry](https://en.wikipedia.org/wiki/Analytical_chemistry) is a method for the quantitative determination of [nitrogen](https://en.wikipedia.org/wiki/Nitrogen) contained in [organic substances](https://en.wikipedia.org/wiki/Organic_compound) plus the nitrogen contained in the inorganic compounds [ammonia](https://en.wikipedia.org/wiki/Ammonia) and [ammonium](https://en.wikipedia.org/wiki/Ammonium) (NH3/NH4+). Without modification, other forms of inorganic nitrogen, for instance [nitrate](https://en.wikipedia.org/wiki/Nitrate), are not included in this measurement (**Kjeldahl, J. 1883).**The Kjeldahl method was developed in 1883 by a brewer called Johann Kjeldahl. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements. It is usually considered to be *the*standard method of determining protein concentration. Because the Kjeldahl method does not measure the protein content directly a *conversion factor (F)*is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration.

**Digestion.** The food sample to be analyzed is weighed into a *digestion flask*and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to C02 and H20. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH4+) which binds to the sulfate ion (SO42-) and thus remains in solution:

N(food) ® (NH4)2SO4 (1)

**Neutralization.** After the digestion has been completed the digestion flask is connected to a *receiving flask*by a tube. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas:

(NH4)2SO4 + 2 NaOH ® 2NH3 + 2H2O + Na2SO4 (2)

The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion:

NH3 + H3BO3 (boric acid) ® NH4+ + H2BO3-(borate ion) (3)

**Titration.** The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.

H2BO3-+ H+ ® H3BO3 (4)

The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original food (Equation 3). The following equation can be used to determine the nitrogen concentration of a sample that weighs *m* grams using a *x*M HCl acid solution for the titration:

https://people.umass.edu/~mcclemen/Proteins1.gif

Where *v*s and *v*b are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. A blank sample is usually ran at the same time as the material being analyzed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis. Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor: %Protein = F %N.

* **Dumas Methods**

The **Dumas method** in [analytical chemistry](https://en.wikipedia.org/wiki/Analytical_chemistry) is a method for the quantitative determination of [nitrogen](https://en.wikipedia.org/wiki/Nitrogen) in [chemical substances](https://en.wikipedia.org/wiki/Chemical_substance) based on a method first described by [Jean-Baptiste Dumas](https://en.wikipedia.org/wiki/Jean-Baptiste_Dumas) in 1826 **(Dr. Julian McClements. D, 2007).**The Dumas technique has been automated and instrumentalized, so that it is capable of rapidly measuring the crude [protein](https://en.wikipedia.org/wiki/Protein) concentration of food samples. This automatic Dumas technique has replaced the [Kjeldahl method](https://en.wikipedia.org/wiki/Kjeldahl_method) as the standard method of analysis for protein content for food and animal feeds

An automated instrumental technique has been developed which is capable of rapidly measuring the protein concentration of food samples. It is beginning to compete with the Kjeldahl method as the standard method of analysis for proteins for some foodstuffs due to its rapidness.

A sample of known mass is combusted in a high temperature (about 900 oC) chamber in the presence of oxygen. This leads to the release of CO2, H2O and N2. The CO2 and H2O are removed by passing the gasses over special columns that absorb them.

The nitrogen content is then measured by passing the remaining gasses through a column that has a thermal conductivity detector at the end. The column helps separate the nitrogen from any residual CO2 and H2O that may have remained in the gas stream.

The instrument is calibrated by analyzing a material that is pure and has a known nitrogen concentration*,* such as EDTA (= 9.59%N).Thus, the signal from the thermal conductivity detector can be converted into a nitrogen content.

As with the Kjeldahl method it is necessary to convert the concentration of nitrogen in a sample to the protein content, using suitable conversion factors which depend on the precise amino acid sequence of the protein.

* **UV-visible techniques**.

A number of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. The basic principle behind each of these tests is similar. First of all, a calibration curve of absorbance (or turbidity) versus protein concentration is prepared using a series of protein solutions of known concentration. The absorbance (or turbidity) of the solution being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration curve. The main difference between the tests are the chemical groups which are responsible for the absorption or scattering of radiation, *e.g.,*peptide bonds, aromatic side-groups, basic groups and aggregated proteins (Latimer, G.W. 2016). A number of the most commonly used UV-visible methods for determining the protein content of foods are highlighted below:

**Direct measurement at 280nm.** Tryptophan and tyrosine absorb ultraviolet light strongly at 280 nm. The tryptophan and tyrosine content of many proteins remains fairly constant, and so the absorbance of protein solutions at 280nm can be used to determine their concentration. The advantages of this method are that the procedure is simple to carry out, it is nondestructive, and no special reagents are required. The major disadvantage is that nucleic acids also absorb strongly at 280 nm and could therefore interfere with the measurement of the protein if they are present in sufficient concentrations. Even so, methods have been developed to overcome this problem, *e.g.,*by measuring the absorbance at two different wavelengths.

**Biuret Method.** A violet-purplish color is produced when cupric ions (Cu2+) interact with *peptide bonds*under alkaline conditions. The biuret reagent, which contains all the chemicals required to carry out the analysis, can be purchased commercially. It is mixed with a protein solution and then allowed to stand for 15-30 minutes before the absorbance is read at 540 nm. The major advantage of this technique is that there is no interference from materials that adsorb at lower wavelengths, and the technique is less sensitive to protein type because it utilizes absorption involving peptide bonds that are common to all proteins, rather than specific side groups. However, it has a relatively low sensitivity compared to other UV-visible methods.

**Lowry Method.** The Lowry method combines the biuret reagent with another reagent (the Folin-Ciocalteau phenol reagent) which reacts with *tyrosine and tryptophan*residues in proteins. This gives a bluish color which can be read somewhere between 500 - 750 nm depending on the sensitivity required. There is a small peak around 500 nm that can be used to determine high protein concentrations and a large peak around 750 nm that can be used to determine low protein concentrations. This method is more sensitive to low concentrations of proteins than the biuret method.

**Dye binding methods.** A known excess of a negatively charged (anionic) dye is added to a protein solution whose pH is adjusted so that the proteins are positively charged (*i.e.*< the isoelectric point). The proteins form an insoluble complex with the dye because of the electrostatic attraction between the molecules, but the unbound dye remains soluble. The anionic dye binds to cationic groups of the basic amino acid residues (histidine, arganine and lysine) and to free amino terminal groups. The amount of unbound dye remaining in solution after the insoluble protein-dye complex has been removed (*e.g.,*by centrifugation) is determined by measuring its absorbance. The amount of protein present in the original solution is proportional to the amount of dye that bound to it: dyebound = dyeinitial - dyefree.

**Turbimetric method.** Protein molecules which are normally soluble in solution can be made to precipitate by the addition of certain chemicals, *e.g.,*trichloroacetic acid. Protein precipitation causes the solution to become turbid. Thus, the concentration of protein can be determined by measuring the degree of turbidity.

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