ASSIGNMENT 8

PGD004 - Post Graduate Diploma in Human Nutrition

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ASSIGNMENT 8

1. Explain the different characteristics of foods are analyzed.

Food Quality can be defined as combinations of attributes or characteristics of a product that have significance in determining the degree of acceptability of that product to the consumer.

It can also be said as measure of purity, strength, physicochemical & oregano-leptic characteristic of food products but the classical definition of quality is composite of these characters that differentiate between individual units of the products & have significance in determining the degree of acceptability of that unit by the user, Three methods;

1. Subjective method
2. Objective method
3. Microscopic method

1. Subjective Method;

Evaluating quality are based on opinion of the investigators. it includes sense organs. It is usually a physiological reaction which is a result of past training, experience of the individual influence of personal preference & power of perception. It is also referred as subjective or sensory method e.g. flavor, color, touch, odour and taste.

2. **Objective methods;** of quality are based on observations from which the human perception is excluded. They are based on scientific tests. Physical methods of Measurement; This is perhaps the quickest method s are generally.

They are concerned with such attributes of product quality as size, texture, color, consistency imperfections or they may be concerned with process variables like headspace, fill, drained weight, or vacuum Chemical methods of measurement.

Standard analysis methods are generally used for quantitative chemical evaluation in most cases, but these chemical analyses are often too long & tedious as a result industries have developed method termed as quick test for such as those for; enzymes, moisture, fiber, PH or acidity

3. Microscopic method

They have excellent application in a quality control programme because they help in determination of microbial count, spoilage protection in fresh and processed products and can differentiate between cell types and organisms.

These methods can be divided into two categories

1. Adulteration & contamination Examination will indicate the presence of molds, insects, excreta or foreign material. Each test is specific.

2. Differentiation between cell type, tissue type, & m/o of various stored foods sensory attributes;

- Appearance; of fresh fruits, fruits & vegetables is one of the first & most important quality parameter made by the buyers / wholesaler /retailer & consumers
- Color; it is perceived when the light is reflected of the commodity surface & falls upon the eyes of the retina. It depends on light and intensity of light. Chemical & physical
- characteristics of the commodity person's ability to differentiate various chemical & physical characteristics of the commodity
- Shape & Size; Uniform & characteristic shape is an important quality characteristic which are susceptible to mechanical injury & generally avoided by the consumer E.g. Compact Broccoli florets are desirable for fresh markets while as fresh cut space between the clusters of florets is important to allow for cutting without injury for market.
- Absence of defects; the product can be evaluated for presence or absence of the defects The level of tolerance for each type of defects such as cuts, bruises, disease symptoms, low temp injury, physiological disorder & should be determined.

Generally, Food quality is the quality characteristics of food that is acceptable to consumers. This includes external factors as appearance (size, shape, colour, gloss, and consistency), texture, and flavor; factors such as federal grade standards (e.g. of eggs) and internal (chemical, physical, microbial).

Food quality is an important for food manufacturing requirement, because food consumers are susceptible to any form of contamination that may occur during the manufacturing process. Many consumers also rely on manufacturing and processing standards, particularly to know what ingredients are present, due to dietary, nutritional requirements (kosher, halal, vegetarian), or medical conditions (e.g., diabetes, or allergies).

1. Minimally processed- some foods are close to the way they naturally occur. Fruits, vegetables, whole grains, dairy, meats, beans, nuts and seeds. These are easier to digest and tend to be free from fake ingredients.

2. Organic- Organic produce is not sprayed with any pesticides or chemicals. There are no artificial substances added, and nothing is genetically modified. Choose organic whenever possible.

3. Contains real ingredients-. A perfect example is "natural flavouring". Sounds good, but do the actually know what it is, They may be surprised to know that these natural flavourings are usually artificial additives that are highly addictive and neurologically toxic.

4. Local- Local produce is always healthier than shipped produce. Many imported foods are picked before they are ripe and are delivered weeks after. The longer fruits and vegetables have been cut off from their life source, the less nutrients they contain.

5. Seasonal- Seasonal food is higher in nutrients than food that is artificially grown out of season. Buying seasonal foods usually means the taste will be much better and your fruits and vegetables will be less expensive.

Characteristics of foods can be summarized under the followings;

1. ***Sensory Quality:*** (colour, flavour, texture, size, shape, appearance, freedom from defects).
2. ***Nutritional Quality:*** Vitamins, minerals, protein, energy as carbohydrate or fat, fibre content. Anti-nutritional quality (pesticide residues, toxins such as heavy metals, naturally occurring anti-nutritional factors such as cyanide complexes in cassava, enzyme inibitors in some vegetables, etc.).
3. ***Quality Expressed as Financial Value:*** This depends in pan on each of the above factors, but more importantly on the demand for a food versus the available supply.
4. ***Microbiological Quality:*** Bacteria, yeasts and moulds, the difference between safe and pathogenic microorganisms, microbial toxins.

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

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents.

These analytical procedures are used to provide information about a wide variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes.

Reasons for Analyzing Foods

Foods are analyzed by scientists working in all of the major sectors of the food industry including food manufacturers, ingredient suppliers, analytical service, government laboratories, and University research laboratories. The various criteria used in the selection of an appropriate technique for foods analysis are briefly discussed below;

Government Regulations and Recommendations

Government regulations and recommendations are designed to maintain the general quality of the food supply, to ensure the food industry provides consumers with foods that are wholesome and safe, to inform consumers about the nutritional composition of foods so that they can make knowledgeable choices about their diet, to enable fair competition amongst food companies, and to eliminate economic fraud. There are a number of Government Departments Responsible for regulating the composition and quality of foods, for example in South Sudan we have the Food and Drug authority of Republic of South Sudan that oversee all the quality of Drugs and Food imported and exported in the country.

So the Government regulations and recommendations is one of the criteria used in selecting an appropriate Technique for food analysis.

Standards

Government agencies have specified a number of voluntary and mandatory standards concerning the composition, quality, inspection, and labeling of specific food products;

Mandatory Standards is another criterion used in selecting an appropriate Technique for food analysis as below;

- *Standards of Identity*. These regulations specify the type and amounts of ingredients that certain foods must contain if they are to be called by a particular name on the food label.

- For some foods, there is a maximum or minimum concentration of a certain component that they must contain.
- *Standards of Quality*. Standards of quality have been defined for certain foods (*e.g.*, canned fruits and vegetables) to set minimum requirements on the color, tenderness, mass and freedom from defects.
- *Standards of Fill-of-Container*. These standards state how full a container must be to avoid consumer deception, as well as specifying how the degree of fill is measured.

Voluntary Standards:

- *Standards of Grade*. A number of foods, including meat, dairy products and eggs, are *graded* according to their quality, *e.g*. from standard to excellent. For example, meats can be graded as prime, choice, select, standard etc according to their origin, tenderness, juiciness, flavor and appearance.
- There are clear definitions associated with these descriptors that products must conform to before they can be given the appropriate label.
- Specification of the grade of a food product on the label is voluntary, but many food manufacturers opt to do this because superior grade products can be sold for a higher price.
- The government has laboratories that food producers send their products too to be tested to receive the appropriate certification. This service is requested and paid for by the food producer.

Nutritional Labeling

Nutritional labels state the total calorific value of the food, as well as total fat, saturated fat, cholesterol, sodium, carbohydrate, dietary fiber, sugars, protein, vitamins, calcium and iron.

The label may also contain information about nutrient content claims (such as low fat, low sodium, high fiber, fat free etc.), although government regulations stipulate the minimum or maximum amounts of specific food components that a food must contain if it is to be given one of these nutrient content descriptors.

Authenticity, the price of certain foods is dictated by the quality of the ingredients that they contain. For example, a packet of premium coffee may claim that the coffee beans are from Columbia, or the label of an expensive wine may claim that it was produced in a certain region, using a certain type of grapes in a particular year.

Food Inspection and Grading

The government has a Food Inspection and Grading Service that routinely analyses the properties of food products to ensure that they meet the appropriate laws and regulations. Hence, both government agencies and food manufacturers need analytical techniques to provide the appropriate information about food properties.

Food Safety

One of the most important reasons for analyzing foods from both the consumers and the manufacturers standpoint is to ensure that they are safe. It would be economically disastrous, as well as being rather unpleasant to consumers, if a food manufacturer sold a product that was harmful or toxic. A food may be considered to be unsafe because it contains harmful microorganisms (*e.g.*, Listeria, Salmonella), toxic chemicals (*e.g.*, pesticides, herbicides) or extraneous matter (*e.g.*, glass, wood, metal, insect matter).

It is therefore important that food manufacturers do everything they can to ensure that these harmful substances are not present, or that they are effectively eliminated before the food is consumed. This can be achieved by following good manufacturing practice regulations specified by the government for specific food products and by having analytical techniques that are capable of detecting harmful substances.

In many situations it is important to use analytical techniques that have a high sensitivity, *i.e.*, that can reliably detect low levels of harmful material. Food manufacturers and government laboratories routinely analyze food products to ensure that they do not contain harmful substances and that the food production facility is operating correctly.

Quality control

The food industry is highly competitive and food manufacturers are continually trying to increase their market-share and profits. To do this they must ensure that their products are of *higher quality*, *less expensive*, and more *desirable* than their competitors, whilst ensuring that they are *safe* and *nutritious*. To meet these rigorous standards food manufacturers need analytical techniques to analyze food materials before, during and after the manufacturing process to ensure that the final product meets the desired standards.

Characterization of raw materials

Manufacturers measure the properties of incoming raw materials to ensure that they meet certain minimum standards of quality that have previously been defined by the manufacturer. If these standards are not met the manufacturer rejects the material. Even when a batch of raw materials has been accepted, variations in its properties might lead to changes in the properties of the final product.

By analyzing the raw materials it is often possible to predict their subsequent behavior during processing so that the processing conditions can be altered to produce a final product with the desired properties. For example, the color of potato chips depends on the concentration of reducing sugars in the potatoes that they are manufactured from: the higher the concentration, the browner the potato chip.

Thus it is necessary to have an analytical technique to measure the concentration of reducing sugars in the potatoes so that the frying conditions can be altered to produce the optimum colored potato chip.

Monitoring of food properties during processing.

It is advantageous for food manufacturers to be able to measure the properties of foods during processing. Thus, if any problem develops, then it can be quickly detected, and the process adjusted to compensate for it. This helps to improve the overall quality of a food and to reduce the amount of material and time wasted. For example, if a manufacturer were producing a salad dressing product, and the oil content became too high or too low they would want to adjust the processing conditions to eliminate this problem.

These techniques allow problems to be determined much more quickly and therefore lead to improved product quality and less waste.

Characterization of final product.

Once the product has been made it is important to analyze its properties to ensure that it meets the appropriate legal and labeling requirements, that it is safe, and that it is of high quality. It is also important to ensure that it retains its desirable properties up to the time when it is consumed.

A system known as **Hazard Analysis and Critical Control Point** (HACCP) has been developed, whose aim is to systematically identify the ingredients or processes that may cause problems (hazard analysis), assign locations (critical control points) within the manufacturing process where the properties of the food must be measured to ensure that safety and quality are maintained, and to specify the appropriate action to take if a problem is identified.

Research and Development

In recent years, there have been significant changes in the preferences of consumers for foods that are healthier, higher quality, lower cost and more exotic. Individual food manufacturers must respond rapidly to these changes in order to remain competitive within the food industry.

Experiments are designed to provide information that leads to a better understanding of the role that different ingredients and processing operations play in determining the overall properties of foods.

Scientists working for food companies or ingredient suppliers usually carry out *product development*.

In both fundamental research and product development analytical techniques are needed to characterize the overall properties of foods (*e.g.*, color, texture, flavor, shelf-life *etc.*), to ascertain the role that each ingredient plays in determining the overall properties of foods, and to determine how the properties of foods are affected by various processing conditions (*e.g.*, storage, heating, mixing, freezing).

Properties Analyzed

Food analysts are interested in obtaining information about a variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes.

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.

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.

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.

The structure of a food can be examined at a number of 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 nm). 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

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

Establishing schedule and cost baselines

The schedule and cost baselines are established only after scope is determined. Without a clear picture of what the project will produce, you cannot determine how long it will take or how much it will cost.

The schedule baseline is the approved project schedule, the basis for measuring and reporting schedule performance. The cost baseline is the approved time-phased budget, against which cost performance will be measured. It's determined by adding the costs for a specific project period or phase, which requires assigning costs to project tasks. To establish schedule and cost baselines:

1. Develop the schedule by identifying the activities and tasks to produce each deliverable in the WBS.
2. Identify resources for each task. Consider constraints or how much time each person can realistically devote to this project.
3. Estimate how long (in hours or days) it will take to complete each task.
4. Estimate the cost of each task, using an average hourly, or daily, rate for each resource, plus any fixed costs associated with the task.
5. Determine which tasks are dependent on others and then develop the critical path.

6. Develop the cost baseline; this is a time-phased budget to measure the project's cost performance. To do this, add the estimated costs, by task or by time period.

Responding to variances: Change control

After you have established scope, schedule, and cost baselines, create the steps the team will take to manage variances for these plans. This information becomes your project change management plan. This plan defines when you determine a project change request (PCR) is required, how to document variances and submit for approval, and what happens after a change request is approved.

Variance calculations are used to determine if a PCR is needed and if the project schedule or cost baselines will be changed. Variances may be either positive or negative:

- A positive variance indicates that the project is ahead of schedule or under budget. Positive scenarios might enable you to reallocate money and resources to those in the negative territory.
- A negative variance is your indicator that the project is behind schedule or over budget and that you need to take action. You might have to increase your budget or accept reduced profit margins.

Variance thresholds are an important component of any project change management plan. They constitute the material changes to the project, and therefore necessitate documentation and approval in a PCR. Not all PCRs will result in reestablishing scope, schedule, or budget. This is a significant task, one that can require considerable time to complete, and you'll be obliged to get approval up and down the project organization.

Tracking cost and schedule variances throughout the life cycle of the project helps you identify weak spots — areas with repeated changes — and respond accordingly. For example, if you see that the testing team is encountering continual delays, you may need to assign additional resources to stay on schedule.

Taking it one step further: Earned-value analysis

No discussion about project variances is complete without mentioning earned value, a project management technique for estimating cost and schedule at a given time. Earned-value analysis compares the work finished with the established baselines. It helps you evaluate current project performance and make course corrections where needed.

Asking the earned-value questions

At any point, earned-value analysis measures project health by asking three key questions:

- Planned value: What is the amount needed for the work?
- Earned value: What did you actually complete?
- Actual cost: How much did it cost you to complete the work?

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

Determination of moisture is important economically to the processor and the consumer. Moisture content of a food product will affect its stability and quality. Moisture is inversely proportional to the amount of dry matter in the food.

The importance of determination of moisture content in food stuff Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons:

Legal and Labeling Requirements. There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.

Economic. The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.

Microbial Stability. The propensity of microorganisms to grow in foods depends on their water content. For this reason many foods are dried below some critical moisture content.

Food Quality. The texture, taste, appearance and stability of foods depends on the amount of water they contain.

Food Processing Operations. A knowledge of the moisture content is often necessary to predict the behavior of foods during processing, e.g. mixing, drying, flow through a pipe or packaging. It is therefore important for food scientists to be able to reliably measure moisture contents.

A number of analytical techniques have been developed for this purpose, which vary in their accuracy, cost, speed, sensitivity, specificity, ease of operation, etc. The choice of an analytical procedure for a particular application depends on the nature of the food being analyzed and the reason the information is needed.

The total water content of food involves the concepts of "free" and "bound" water, equilibrium moisture content, moisture adsorption, moisture desorption etc. The most important term is "bound" water on which the ultimate accuracy of a method for moisture content determination is related.

Bulk water: - Bulk water is free from any other constituent so that each water molecules is surrounded only by other water molecules. It therefore, has physico-chemical properties that are the same as those of pure-water, e.g, melting point,boiling point, density, compressibility, heat of vaporization electron magnetic absorption spectra.

Capillary or trapped water: - Capillary water is held in narrow channels capillary forces. Trapped water is held within spaces within a food that are surrounded by a physical barrier that prevent the water molecules from easily escaping e.g, an emulsion droplet or a biological cell. The

majority of this type of water is involved in normal water, water bonding and so it has Physico- chemical properties similar to that of bulk water.

Physically bonded water: - A significant fraction of the water molecule in many foods is not completely surrounded by other water molecules, but is in molecular contact with other food constituent e.g. protein, carbohydrate or minerals. The bonds between water molecules and these constituents are often significantly different from normal water, water bond and so this type of water has different physico-chemical properties than bulk water e.g. melting point, boiling point, density, compressibility, heat of vaporization, electro-magnetic absorption spectra.

Chemically bonded water: - some of the water molecule present in a food may be chemically bonded to other molecules as water of crystallization is as hydrate e.gNaS04.10H20.

Forced Oven Draft—Sample is rapidly weighed into a moisture pan and placed in the oven for an arbitrarily selected time if no standard method exists. Drying time periods for this method are 0.75- 24 hours, depending on the food sample.

Vacuum Oven—Drying under reduced pressure (25-100 mm Hg) allows a more complete removal of water and volatiles with-out decomposition within 3-6 hr drying time.

Microwave Oven—A precise and rapid technique that allows some segments of the food industry to make in-process adjustments of moisture content before final packaging. In vacuum microwaves, a drying time of 10 minutes can yield results equivalent to those of five hours in a standard vacuum oven. Advantages of the oven-drying and vacuum oven-drying methods are their easy handling and possibility for simultaneous determinations.

This technique is particularly suited to food products that show erratic results when heated or submitted to a vacuum. It is the method of choice for low-moisture foods such as dried fruits and vegetables, candies, chocolate, roasted coffee, oils and fats, and low-moisture foods high in sugar or protein.

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. 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.

- Electric (dielectric or conductivity)—Moisture content is determined by measuring the change in capacitance or resistance to an electric current passed through a sample.
- Hydrometry—Used to determine moisture/ solid content of beverages and sugar solutions. Measuring the specific gravity or density of the sample via one of the following instruments:
- Pycnometer: used to compare the weights of equal volumes of a liquid and water. Yields density of the liquid compared to water.
- Hydrometer: a standard weight on the end of a spindle which displaces a weight of liquid equal to its own weight. In a low-density liquid, weight will sink to a greater depth.
- Westphal Balance: functions on the principle that the plummet on the balance will be buoyed by the weight of liquid equal to the volume displaced.

- Refractometry—Measures moisture content of oils and syrups as a function of the degree of refraction of a light beam as it passes through the sample.
- Infrared Analysis—Measures the energy that is reflected or transmitted by the sample when exposed to infrared light.
- Freezing Point—Measures the solutes present by determining the freezing point of the sample. Used principally to measure for added water content 1.2 oven drying method for the thermally stable products. Theory Moisture % = weight loss *100 Weight of the sample Total solid % =100-moisture

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

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Analytical Methods for Lipid Oxidation Measurements

Measurement of lipid oxidation can be carried out by a wide range of methods such as peroxide value (PV), anisidine value (AV), thiobarbituric acids reacting substances (TBARS) as well as instrumental methods such as HPLC, GC-MS, NIR, FTIR and DSC. Sensory evaluation of oxidative flavor deterioration is another important method that should always be included at some stage to understand how lipid oxidation has impacted sensory properties of the food product in question.

In lipid oxidation studies on meat products there seems to be a fairly good correlation between TBARS and sensory data, although in many cases no attempts have been made to statistically evaluate the correlations (Eckert et al., 1997; Murano et al., 1998; Winne and Dirinck, 1997). However, newer data indicate that even in meat TBARS may be an unreliable method (Summo et al., 2010).

This may be related to the fact mentioned above that lipid oxidation has several alternate pathways. Therefore, if lipid oxidation is only followed by measuring formation of TBARS and peroxide values the major proportions of oxidation products may be missed and the extent of lipid oxidation may be significantly underestimated.

This type of co-oxidation will thus consume lipid oxidation intermediates and products and at the same time other substrates in the food system will be affected by oxidation. If only the traditional lipid oxidation measurements are performed, lipid oxidation can be underestimated, when it is in fact spread to other components of the food system, which may have an effect on sensory properties of the food product.

Good correlations data from headspace GC analysis and sensory data have been found for a range of different oxidized products such as fish (Milo and Grosch, 1995), fish oil enriched milk (Venkateshwarlu et al., 2004), mayonnaise and dressing (Hartvigsen et al., 2000; Let et al., 2007a) and boiled potatoes (Blanda et al., 2010). Headspace GC analysis can therefore be recommended as one of the best methods to chemically assess oxidative flavor deterioration. Different headspace methods are available for collection of the volatile oxidation products including static headspace, dynamic headspace, purge and trap and solid phase microextraction. Thomsen et al. (2016) have recently compared the performance of some of these methods in neat oil and two emulsified systems.

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

Protein Analysis

Kjeldahl method: In the Kjeldahl procedure, after digestion in concentrated sulfuric acid, the total organic nitrogen is converted to ammonium sulfate. Ammonia is formed and distilled into boric acid solution under alkaline conditions. The borate anions formed are titrated with standardized hydrochloric acid, by which is calculated the content of nitrogen representing the amount of crude protein in the sample.

Protein Determination - Kjeldahl

The Kjeldahl method is used worldwide as the standard for analyzing protein content in food, feed, feed ingredients and beverages. Typical analyses are protein in corn, barley wheat, seeds and protein in milk, beer, cookies, sausage, meat and flour.

Kjeldahl Digestion

In the Kjeldahl method the sample is boiled in sulphuric acid together with salt and catalyst. This very reactive and corrosive mixture puts high demands on the materials used. Also a well designed fume

removal system is necessary to avoid damages on the laboratory inventories. An Exhaust together with a Scrubber is often the solution for this.

Kjeldahl Analysis

Analyses where it is important to perform the titration simultaneously with the distillation e.g. Kjeldahl put high demands on the titration system. The colorimetric detection of the end point is often the only possibility. Even tougher it becomes if the Nitrogen level varies a lot from sample to sample, then the unique Predictive Titration System improves the function.

Protein Analysis

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Biuret method: Under alkaline conditions, peptide bonds react with cupric ions to produce a violet-purplish color, the intensity of which at 540 nm is correlated to the protein content of the sample (Owusu-Apenten, 2002). This method is quick and simple with little interference from nonpeptide or nonprotein sources.

Lowry method: The Lowry method is developed based on the biuret method. Proteins react with Folin–Ciocalteu phenol reagent to produce a blue conjugate at 750 nm. The intensity of color is proportional to the protein content of the sample (Owusu-Apenten, 2002). Although this method is very sensitive, it is interfered by sucrose and lipids.

Ultraviolet 280 nm Absorption Method: Proteins, at 280 nm, show strong absorption primarily for tryptophan and tyrosine residues. This technique is mainly applied in a purified protein system, because absorbance will be falsely increased if particulates or other substance like nucleic acids are in the solution (Scopes, 1994). This method is noninvasive; thus samples can be used for other analyses after protein determination.

Other methods: In addition to the aforementioned methods, the Dumas, IR spectroscopy, bicinchoninic acid, anionic dye-binding, Bradford dye-binding, Ninhydrin, and immunological methods are some other techniques commonly used for protein analysis (Nielsen, 2010b; Owusu-Apenten, 2002; Ozaki *et al.*,

2006). Choosing an appropriate method depends on the properties of proteins present in the samples as well as the characteristics of analytical approaches.

The nutritional value of a protein is determined by the amino acid composition and the digestibility of that protein. The most common parameters for protein quality are the AAS, BV, TD, and NPU. The procedure for calculation is further elaborated in the reference Owusu-Apenten (2002).

Kjeldahl method

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.

Principles

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, , 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 $C0_2$ and H_20. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution:

N(food) □ $(NH_4)_2SO_4$ (1)

Neutralization

After the digestion has been completed the digestion flask is connected to a *recieving 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:

$(NH_4)_2SO_4 + 2\ NaOH$ □ $2NH_3 + 2H_2O + Na_2SO_4$ (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: $NH_3 + H_3BO_3$ (boric acid) $\rightarrow$ $NH_4^+ + H_2BO_3^-$ (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.

$H_2BO_3^- + H^+ \rightarrow H_3BO_3$ (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:

Advantages and Disadvantages

Advantages. The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision and good reproducibility have made it the major method for the estimation of protein in foods.

Disadvantages. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The use of concentrated sulfuric acid at high temperatures poses a considerable hazard, as does the use of some of the possible catalysts The technique is time consuming to carry-out.

Enhanced Dumas method

Recently, an automated instrumental technique has been developed which is capable of rapidly measuring the protein concentration of food samples. This technique is based on a method first described by a scientist called Dumas over a century and a half ago.

General Principles

A sample of known mass is combusted in a high temperature (about 900 ^{o}C) chamber in the presence of oxygen. This leads to the release of CO_2, H_2O and N_2. The CO_2 and H_2O 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.

Advantages and Disadvantages

Advantages: It is much faster than the Kjeldahl method (under 4 minutes per measurement, compared to 1-2 hours for Kjeldahl). It doesn't need toxic chemicals or catalysts. Many samples can be measured automatically. It is easy to use.

Disadvantages: High initial cost. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The small sample size makes it difficult to obtain a representative sample.

Methods using UV-visible spectroscopy

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.

A number of the most commonly used UV-visible methods for determining the protein content of foods are highlighted below:

Reference.

1. *B. Jiang, ... M. Miao, in Encyclopedia of Agriculture and Food Systems, 2014*
2. Mayounga, A. T. (2018) "Antecedents of recalls prevention: analysis and synthesis of research on product recalls." Supply Chain Forum: An International Journal, 19(3). https://doi.org/10.1080/16258312.2018.1530575. Retrieved 2018-11-23