A Food Technology Lab Manual

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Chapter 1

Introduction to Laboratory Practices

A laboratory is a workshop for a scientist. Here researcher does the techniques for preparation of chemical substances and formulate new methods. She/he must know what all procedures are involved in the experiment and what all equipments and chemicals are required for it. Proper knowledge about the working principles of the equipment and the nature of the chemicals are essential.

Instruction to Work in the Laboratory

1. Lab work is always totally different from any kind of office work. A researcher is not absolutely free from research thoughts at any time of the day. In that case, punctuality is of top priority for him/her.
2. Your attitude towards your work reflects the results you get.
3. Irresponsibility in any manner won't be tolerated.
4. Always keep your lab environment neat and tidy.
5. Chemicals, glassware and all lab belongings should be placed in the space provided.
6. Keep your working bench clean of everything. Never keep books, purses, bags, etc. on the working bench. Nothing should be lying on the bench.
7. Don't eat or drink or talk while working in the lab. Your talk must be related to your work assigned.
8. You must have a practical basic record, field book, a pen or pencil, a laboratory coat, a head cap, a mask, a lab slipper and gloves to work in the lab.
9. Record your results at time. For any difficulty, ask your laboratory in charge.
10. Record every single calculation in your field book and every step involved in the procedure.
11. Plan your work in order to finish it in stipulated time.
12. Be economical with every resource/reagents. Only small quantities of the reagents should be used.
13. Handle the glass equipments carefully. If it breaks report it to the lab in charge.
14. Dispose all the waste liquids in the sink; allow water to run for some time by opening the water tap.
15. Never spill any chemicals in or on the lab equipment. If so clean the equipment after its use.
16. All the electric supplies must be plugged out if not in use.
17. Lights, fan, a/c and computer system should be off if not required.
18. Water supplies should be closed tightly after use.
19. You should save electricity and water at least for your future needs.
20. In case of any injury or burns go for a medical assistance with the first aid box provided in the laboratory.
21. Most importantly you should maintain a good relationship with your colleagues for their valuable support. Working with healthy groups always improves your scientific knowledge.
22. You should be up to date with the recent trends and findings at least in your field of work.

Basic Record and Field Book/Lab Book

They are the permanent record of your work and hence should contain all the works related to the project. Basic Record is the whole systematic record of the work/study/project in full detail. Field book/lab book is for your daily use in the lab/field and for rough works, calculations, plan schedules, memoirs, etc. You should record your work in these books systematically and regularly. All the experiments conducted in the lab must be recorded in these books. It is a compilation of whole work done by the researcher, so it must be well maintained. Also it can be a good reference book for those who come along. These are the

property of the research station and hence you are not supposed to keep those books in your home. When you resign from the job you should submit them up-to-date to the lab in charge without any delay.
You should note the following points while dealing with field book.

1. Keep the book neat and tidy.
2. Utilize the book efficiently preserving the legibility of your writing.
3. Name of the experiment should be entered along with the date of carrying out that experiment.
4. Next you mention the requirements for the experiment.
5. Summarize the theory and principle. This should be followed by the procedure.
6. Mention the general calculations for the experiment. It should contain all the related works of the project for which it is meant to.

The following points are to be taken care of:

1. Do not tear pages from the field book. Number the pages of field book.
2. Do not over write if a mistake has been committed in recording, put a line over it and write the correct word again.
3. Complete the index, indicating the experiment, its serial number, page number on which it is written.
4. The notebook should always be up to date and may be collected by the lab in charge at any time.
5. You have to submit the field book and basic record at the end of every month on the date assigned.

Mandatory Details Required the Basic Record

1. Index: An index containing the title of each experiment with page number and Sl. No.
2. Brief title of the experiment and date: Every experiment should have a descriptive title.
3. Aim/Objective: A clear objective should be there.
4. Technical Programme: This section should include any materials required, reagent composition, protocol and formulae. Procedure in the form of flow charts is helpful if it involves several parts. If an experiment is a repeat of an earlier experiment, you do not have to write down each step, but can refer to the earlier experiment by page or experiment number. If you make any changes, note the changes and reasons why.
5. Observations: Periodical or quantitative or qualitative observations
6. Results: This section should include the final result of the experiment in accordance with the aim, organized in statistically valid tables and figures and discussed logically and justifiably. All raw data, including gel photographs, printouts, graphs, autoradiographs, etc if present are to be included.
7. Inference: The results obtained should be interpreted in accordance with the principle of the experiment.
8. Future Line: This section includes any suggestions from the protocol done, any refinements required etc.

It is mandatory to have clear and accurate records of all experiments conducted in the laboratory.

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Chapter 2

Introduction to Food Lab

Laboratories are becoming very common in every university and research institutes and a good laboratory is extremely important as many of the equipment are very expensive and sensitive. Hence familiarity with equipment becomes very crucial before starting any experiments.

Food Laboratory

The level I laboratory will carry out the following analysis:-

a) Physical analysis
b) Chemical analysis
c) Microbiological analysis
d) Rheological analysis
e) Functional testing
f) Basic nutrient analysis such as fat, protein, calorific value
g) Sensory analysis

The level II laboratory will carry out the analysis covered in Level 1 Food Laboratory as well as the following analysis:-

a) Contaminants (chemical, microbiological)
b) Toxic substances
c) Pesticides residues
d) Antibiotics and pharmacologically active substances
e) Irradiation of food
f) Detailed nutrient analysis
g) Molecular analysis (genetically modified food)

For the purpose of the recognition, the relevant definitions given in FSS Act, 2006 applies and are reproduced below:-

Adulterant means any material which is or could be employed for making the food unsafe or substandard or misbranded or containing extraneous matter.

Contaminant means any substance, whether or not added to food, but which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry or veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination and does not include insect fragments, rodent hairs and other extraneous matter.

Extraneous matter means any matter contained in an article of food which may be carried from the raw materials, packaging material or process systems used for its manufacture or which is added to it, but such matter does not render such article of food unsafe.

Food means any substance, whether processed, partially processed or unprocessed, which is intended for human consumption and includes primary food to the extent defined in clause, genetically modified or engineered food or food containing such ingredients, infant food, packaged drinking water, alcoholic drink, chewing gum, and any substance, including water used into the food during its manufacture, preparation or treatment but does not include any animal feed, live animals unless they are prepared or processed for placing on the market for human consumption, plants prior to harvesting, drugs and medicinal products, cosmetics, narcotic or psychotropic substances.

Food Additive means any substance not normally consumed as a food by itself or used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such food but does not include contaminants or substances added to food for maintaining or improving nutritional qualities.

Food Safety means assurance that food is acceptable for human consumption according to its intended use.

Hazard means a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Ingredient means any substance, including a food additive used in the manufacture or preparation of food and present in the final product, possibly in a modified form.

Sample means a sample of any article of food taken under the provision of this act or any rules and regulations made thereunder.

Standard means standards notified by food authority.

Unsafe food means an article of food whose nature, substance or quality is so affected as to render it injurious to health.

FUNCTIONS OF FOOD LABORATORY

The Food laboratory shall carry out the following functions, namely:

- Analysis of samples of food sent by any officer or authority authorized by the Food Authority for the purpose and submission of the certificate of analysis to the authorities concerned.
- Investigation for the purpose of fixation of standard of any article of food.
- Investigation in collaboration with the laboratories of Food analysts in the various States and such other laboratories and institutions which the Food Authority may approve on its behalf, for the purpose of standardizing methods of analysis.
- Ensuring that the laboratory follows the scientific protocols laid down for handling/testing the articles of food.
- Maintaining high standards of accuracy, reliability and credibility in the operation of the laboratory and achieving and maintaining the required levels of accreditation and reliability.
- Laying down mechanism for ensuring that personnel of the laboratory adhere to high professional standards and discipline.
- Capacity building by way of organizing professional training, workshops and seminars for the Food analyst, laboratory personnel in the states specified by the Food authority.
- The laboratory should have R&D Capabilities for the purpose of developing standards for any article of food and standardizing methods of analysis.
- The laboratory should have training centre for capacity building by way of organizing professional training, workshops and seminars for the food.

INFRASTRUCTURE

The Applicant Laboratory must have all the infrastructure and facilities required for carrying out the analysis as per the scope applied for.

- Food laboratories shall be designed to meet the testing requirements. The layout of laboratory shall be arranged in such a way that test procedures can be carried out in sequential manner and risk of contamination can be reduced.
- The space shall be managed with respect to specialized activities such as separate space for wet analysis and dry analysis.
- The laboratory layout shall meet the instructions of equipment's and instruments handling.
- Special provisions / rooms shall be available for weighing balance, sensitive instruments, radioactive material, media preparation, storage (samples and chemicals) and washing of glassware.
- Provision of water (potable & distilled), cupboards, sinks, dustbins, reagent shelves, glassware, fume cupboards and power.
- Ventilators and fume cupboard shall be placed carefully such as to maintain the dust free environmental conditions in laboratory and to avoid risk of contamination of test samples/ chemicals.

- Work place shall be smooth, easy to clean and shall be made of material according to the test requirements so as to prevent hazard to laboratory staff.
- Appropriate environmental monitoring programme shall be carried out such as temperature and humidity control. Their daily record shall be maintained. If required positive pressure and laminar flow can be provided.
- Laboratory shall be prohibited from eating, drinking and smoking. Visitors shall be restricted in laboratory especially in contamination prone areas.
- Appropriate system/device shall be used to control environmental contamination.
- Staff shall be aware of Contamination prone areas. They shall be trained to take appropriate measures for safety and security. Analyst shall wear lab coat, gloves, mask and eye shield/goggles while working in lab.
- Laboratory shall follow good housekeeping activities such as floor and wall cleaning, washrooms, dustbins, fumehoods, freezers, refrigerators, air conditioners, air filters, flies killer lamp.
- The laboratory shall be liable to maintain safety measures and pest control measures.

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Chapter 3

Laboratory equipment and their usage

Calibration

Laboratories can get their equipment calibrated from national metrology laboratory in the knowledge that the calibration is internationally traceable. Calibration certificates shall be maintained for volumetric and mass measuring equipment. Calibration shall be performed using reference material and results shall be verified from standards and documented. The records shall mention the date of calibration, reference standard used, next due date of calibration and periodicity of calibration.

List of equipment which can be used in food analysis is as follows:-

- Autoclave
- Centrifuge
- Chromatography assembly/ analyzer
- Colony counter
- Crude fiber assembly
- Deep freezer
- Distillation assembly
- Homogenizer
- Hunter color lab
- Incubator
- Kjeldahl apparatus
- Laboratory hoods
- Laboratory sterilization equipment
- Laminar flow
- Microscope
- Mixer Grinder
- Moisture Analyzer
- Muffle furnace
- Oven
- PH Meter
- Refractometer
- Refrigerator
- Rheometer
- Rotary Evaporator
- Shaking Incubator
- Soxhlet apparatus
- Texture analyzer
- Titration assembly
- UV-Vis Spectrophotometer
- Viscoanalyzer
- Viscometer
- Water activity meter
- Weighing Balance (sensitivity according to the requirements)
- Any other equipment as per requirement

Equipment: Principle and Working

Equipment Features

1. Autoclave

General Description: An autoclave is a large pressure cooker. It is a *moist sterilization* unit.

Principle: It operates under the principle of steam under pressure as the sterilizing agent. High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization (the amount of heat required to convert boiling water to steam).

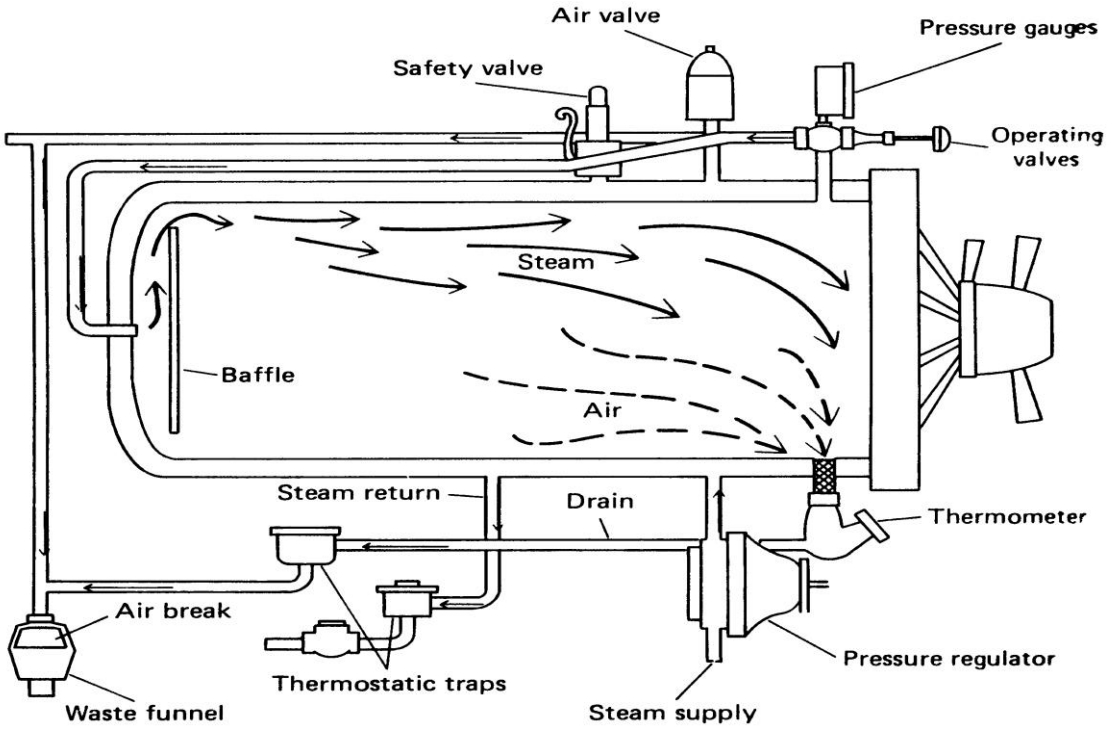
Steam is able to penetrate objects with cooler temperatures because once the steam contacts a cooler surface; it immediately condenses to water, producing a concomitant 1, 870 fold decreases in steam volume. This creates negative pressure at the point of condensation and draws more steam to the area. A condensation continues so long as the temperature of the condensing surface is less than that of steam; once temperatures equilibrate, a saturated steam environment is formed. Achieving high and even moisture content in the steam-air environment is important for effective autoclaving. The ability of air to carry heat is directly related to the amount of moisture present in the air. The more moisture present, the more heat can be carried, so steam is one of the most effective carriers of heat. Steam therefore also results in the efficient killing of cells and the coagulation of proteins.

Autoclave

Moist heat is thought to kill microorganisms by causing coagulation of essential proteins. Another way to explain this is that when heat is used as a sterilizing agent, the vibratory motion of every molecule of a microorganism is increased to levels that induce the cleavage of intramolecular hydrogen bonds between proteins. Death is therefore caused by an accumulation of irreversible damage to all metabolic functions of the organism. Death rate is directly proportional to the concentration of microorganisms at any given time. The time required to kill a known population of microorganisms in a specific suspension at a particular temperature is referred to as thermal death time (TDT). All autoclaves operate on a time/temperature relationship; increasing the temperature decreases TDT, and lowering the temperature increases TDT.

Standard temperatures/pressures employed are 115°C/10 psi, 121°C/15 psi and 132°C/27 psi (psi=pounds per square inch).

Working: Steam enters the chamber jacket, passes through an operating valve and enters the rear of the chamber behind a baffle plate. It flows forward and down through the chamber and the load, exiting at the front bottom. A pressure regulator maintains jacket and chamber pressure at a minimum of 15 psi, the pressure required for steam to reach 121°C (250°F). Overpressure protection is provided by a safety valve. The conditions inside are thermostatically controlled so that heat (more steam) is applied until 121°C is achieved, at which time the timer starts, and the temperature is maintained for the selected time.



Points to Remember: Please note that after loading and starting the autoclave, the processing time is measured after the autoclave reaches normal operating conditions of 121°C (250°F) and 15 psi pressure, not simply from the time you push the "on" button. Due to the fact that autoclaves utilize steam, heat and pressure the risk of personal exposure and potential harm is great. Personnel should wear proper personal protective equipment, i.e. heat resistant gloves, eye protection and a lab coat, particularly when unloading the autoclave. Regularly inspect the autoclave for proper operation. Do not assume that the temperature and pressure is down before opening the chamber. Look at the gauges. Even if the pressure gauge shows "0", open the chamber carefully; crack the door to allow steam to dissipate (don't fling the door open, as steam might come out and burn you). After opening the door, let items sit for five minutes before handling. This will reduce the chance of boil-over and burns. Never place sealed containers in an autoclave they might explode. This allows for expansion during the cycle. Caps must be slightly loose so that pressure created during the cycle does not cause the vessel to break. For screw-cap containers, you can make the lid hand tight and then loosen the lid by one-half turn. Always leave a few inches of "head room" in your containers. That way, if the item boils, it won't spray out into your face. Liquids to be autoclaved must be in an autoclavable vessel that is at least twice as large as the volume to be autoclaved (i.e. If you are autoclaving 1 liter of media, you need to put it in a flask that hold at least 2 liters). Always autoclave the media in a pan to contain spills. Agar will clog the drain in the autoclave and break it.

Do not autoclave items containing solvents, volatile or corrosive chemicals (phenol, trichloroacetic acid, ether, chloroform, etc.) or any radioactive materials.

2. Hot Air Oven

General Description: It is a *dry heat sterilization* unit. A dry heat cabinet is easy to install and has relatively low operating costs; it is nontoxic and does not harm the environment and it is noncorrosive for metal and sharp instruments.

Principle: Sterilization by dry heat is accomplished through conduction. The heat is absorbed by the outside surface of the equipment, and then passes towards the centre of it, layer by layer. The entire system will eventually reach the temperature required for sterilization. Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores. The most common time-temperature relationships for sterilization with hot air sterilizers are:
170°C (340°F) for 60 minutes, 160°C (320°F) for 120 minutes, and 150°C (300°F) for 150 minutes or longer depending upon the volume.

Working: There are two types of dry-heat sterilizers: one is the static-air type and the other is forced-air type. The static-air type is referred to as the oven-type sterilizer as heating coils in the bottom of the unit cause the hot air to rise inside the chamber via gravity convection. This type of dry-heat sterilizer is much slower in heating, requires longer time to reach sterilizing temperature, and is less uniform in temperature control throughout the chamber than the forced-air type.

Points to Remember: Dry heat sterilization technique requires longer exposure time (1.5 to 3 hours) and higher temperatures than moist heat sterilization. Dry heat ovens are used to sterilize items that might be damaged by moist heat or that are impenetrable to moist heat (e.g., powders, petroleum products, sharp instruments).

Hot Air Oven

The Hot air oven is mounted on four rubber foots to prevent slipping and this protects the bench surface. The control panel houses a main ON/OFF switch indicator lamp and temperature setting knob. The scale is calibrated in 5°C steps.

Working Principle of Hot Air Oven is the forced circulation of hot air inside the chamber of oven. As it is a universal scientific fact that in any chamber hot air rises above, so by utilizing this principle when the hot air reaches the top of chamber it is circulated back to bottom by a fan installed inside the chamber and hence optimum amount of heat is achieved gradually inside the Hot Air Oven.

3. Water Still

Water Still

General Description: In this equipment liquid is vaporized (turned to steam), recondensed (turned back into a liquid) and collected in a container.

Principle: The separation of components from a liquid mixture *via* distillation depends on the differences in boiling points of the individual components. Also, depending on the concentrations of the components present, the liquid mixture will have different boiling point characteristics. Therefore, distillation process depends on the vapour pressure characteristics of liquid mixtures. A liquid boils when its vapor pressure equalizes with the surroundings.

4. pH Meter

pH Meter

General Description: This equipment measures pH of the solutions and the tissue culture media. Appropriate buffers stabilize the pH of the electrode and the machine reads the pH of the solution in complement with the buffer.

Principle: Buffer is a solution whose pH does not change very much when small amounts of acid (H+) or base (OH–) are added. This does not mean that no change occurs, only that it is small compared to the amount of acid or base added; the more acid or base added, the more the pH will change. Buffer solutions consist of a conjugate acid-base pair (weak acid plus its salt or weak base plus its salt) in approximately equal amounts (within a factor of 10). Thus, buffers work best at pH within 1 pH unit of the pKa. The concentration of a buffer refers to the total concentration of the acid plus the base form. The higher the concentration of the buffer, the greater its capacity to absorb acid or base. Most biological buffers are used in the range of 0.01–0.02 M concentration. The ratio of the 2 components and the pKa of the acid component determine the pH of the buffer.

$$pH = pKa + \log[\text{base form}]/[\text{acid form}]$$

If everything is behaving ideally, the pH should not depend on the buffer concentration or the presence of other ions in solution. In reality, some buffers do better at this than others. It's best to check the pH of the final solution when preparing buffers from concentrated stocks. Temperature will also affect pH since pKa values, like other equilibrium constants, change with temperature. Again, it's best to check the pH of the buffer at the temperature it will be used.

The most commonly used electrode is made from borosilicate glass, which is permeable to H^+, but not to other cations or anions. Inside is a 0.1 M HCl solution; outside there is a lower H^+ concentration; thus the passage of H^+ from inside to the outside. This leaves negative ion behind, which generates an electric potential across the membrane.

$$E = 2.3 \times RT/F \times \log [H^+]1/[H^+]2$$

where R = gas constant, T = absolute temperature, F = Faraday constant, $[H^+]1$ and $[H^+]2$ are the molar H^+ concentrations inside and outside the glass electrode.

A reference electrode (pH-independent and impermeable to H^+ ions) is connected to the measuring electrode. Reference electrode contains Hg-Hg_2Cl_2 (calomel) paste in saturated KCl. The concentration of 0.1 M HCl (inside the measuring electrode) may decrease by repeated use. Therefore the pH meter has to be standardized against a solution of known pH.

Working:

- Turn on pH Meter. Lift up the electrode and clean the electrode tip by pressing with tissue paper.
- Calibrate using buffer 4±0.01 and buffer 7±0.01
- The buffers should come to the room temperature before calibration.
- Place the electrode in the solution to know the pH.
- If there is an increase in pH, stabilize it with adding 0.1N HCl which lowers the pH. If there is a decrease in pH, stabilize it with adding 0.1N NaOH which increases the pH.
- Add acid or alkali drop by drop and always stir well the solution with glass rod after each addition.
- Read the pH when √A appears.

Points to Remember:

1) Always keep the electrode dipped in buffer 4. Never leave the electrode dry.
2) Be cautious about the electrode level displayed on the screen. It symbolizes the fitness of electrode. Any kind of unusual appearance can cause pH fluctuation.
3) Buffer solutions should always be clear without any turbidity or mycelia growth. Same is the case if the buffer is used to protect electrode.
4) Always prepare fresh buffer solutions every month for calibration.
5) Always prepare buffer solutions in sterile water in the same method mentioned the buffer capsule box.
6) Never mishandle the electrode like using it for stirring the solutions.
7) Always use sterile water to clean the electrode after dipping it in solutions.
8) Never place the electrode in solutions which are in extreme temperature conditions.

9) No hurry should be there for reading the pH.
10) Do not forget to make entry with appropriate comments in the log book.
11) The equipment and its premises should be clean if there is any spill.


Electronic Weighing Balance

5. Electronic Weighing Balance

Principle & Working: Electronic weighing balance accurately measures the weight of chemicals. Calibrate the balance by internal calibration. Place the weighing boat and tare the weight. Wait till it becomes zero. Chemical should be weighed slowly according to the need. Wait till the symbol "g" stabilizes next to the weight shown.
Points to Remember: Always have the knowledge about the maximum and minimum quantity which can be weighed using the balance. Never spill the chemicals on the weighing pan, if it happens wipe off with tissue. Switch off fan and windows nearby when working with it as it may cause fluctuations in the value due to interaction with air density. Always use weigh boats or butter paper to weigh.

6. Laminar Air Flow Chamber

General Description: It provides clean air to the working area, a constant flow of air out of the work area to prevent room air from entering. The air flowing out from the hood suspends and removes contaminants introduced into the work area.
Principle: The most important part of a laminar flow hood is a high efficiency bacteria-retentive filter. Room air is taken into the unit and passed through a pre-filter to remove gross contaminants. The air is then compressed and channeled up behind and through the HEPA filter (High Efficiency Particulate Air filter) in a laminar flow fashion; that is the purified air flows out over the entire work surface in parallel lines at a uniform velocity. The HEPA filter removes nearly all of the bacteria from the air.
Working: Switch on. Let the blower and light on. Wipe with 70% spirit in a vertical manner. Place all the materials required to work in the hood. All should be Spirit sterilized before placing in the LAF. Switch on the UV light for 45 minutes. Let the blower run continuously for 15 minutes. When this time has passed repeat the wipe out of the sterile area with an alcohol soaked piece of absorbent cotton. For cutting explants use a Petri dish (made of glass) or sterile steel plate which should be cleaned with an alcohol soaked piece of absorbent cotton.
Points to Remember:

- A direct path must be maintained between the filter (99.99% at 0.3microns) and the area inside the hood where the manipulations are being performed. Air downstream from non-sterile objects (such as solution containers, hands etc.) becomes contaminated from particles blown off these objects.
- The hands should never obstruct airflow around the area where the needle enters the vial or culture bottle or culture. Also, when pulling the forceps, the fingers should not come in contact with any part of the culture.
- Always minimize time lag: Waste and other items should never enter the hood. All calculations should be done before entering the hood.
- Wash hands and arms before compounding or re-entering the hood. Also, remove any jewelry from the hands and wrists. It is important that you keep your hands within the cleaned area of the hood as much as possible. Do not touch your hair, face or clothing.

- Excess dust should be removed from items before introducing them into the hood.
- Arrange objects in a manner to get full benefit of the laminar flow of air. Critical items should be placed as close to the air source as possible. In a horizontal hood, items should be placed no closer than 3 inches from the very back of the hood (nothing should touch the filter). Occasionally, you may stack a few items however they must be stacked from lower to higher starting from the back of the hood.
- When working in a horizontal laminar flow hood, all work must be performed at a distance of no less than 6 inches from the front edge of the work surface. At a distance of less than 6 inches, laminar flow air begins to mix with the outside air and contamination is possible. Never become so engrossed in your work that you forget this basic rule.
- Avoid spraying or squirting solutions onto the HEPA filter. Always aim away from the filter when opening cultures.
- Outer pouches and wraps should be removed at the edge of the work area as the sterile contents are pulled into the work area. Never bring these items into the main work area.
- Large objects should never be placed near the back of the hood. Not only do these objects contaminate everything downstream, but they also disrupt the laminar flow pattern of air which normally suspends the contaminants and removes them from the area.
- Remember that hand cleanliness is further reduced each time more bottles and other non-sterile items are handled.
- Before and after inoculation and in intervals, the work surface of the laminar flow hood should be thoroughly cleaned with 70% alcohol. A long side to side motion should be used starting at the back of the hood and then working forward. The acrylic plastic sides should also be cleaned periodically.
- It is possible to overcome the established airflow velocity by a strong reverse current produced by coughing, quick movements, talking etc. Keep all of these to a minimum in order to maintain a sterile environment. Do not talk, cough or sneeze into the hood.

Orbital Shaker

7. Orbital Shaker

General Description: This is mainly used to provide for gentle and intensive mixing of biological and chemical compounds in a laboratory.

Working: It is a table-top laboratory instrument applicable for extracting, dissolving slow-reacting samples; cultivation of cells; extraction of mineral oil of soil, of tissue culture for analytical diagnostics; de-aeration of tested biodegradable materials and samples; rotating closed containers for dialysis.

Cooling Centrifuge

8. Centrifuges

General Description: A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity and rotor speed. In biology, the particles are usually cells, subcellular organelles, viruses, large molecules such as proteins and nucleic acids.

Principle: Centrifuge works on the basic Theory of Sedimentation. Molecules separate according to their size, shape, density, viscosity, and centrifugal force. The simplest case is a spherical molecule. If the liquid has the density of "do" and the molecule has a density of "d", and if d>do, then the protein will

sediment. In gravitational field, the motor force (Pg) equals the acceleration of gravity (g) multiplied by the difference between the mass of the molecule and the mass of a corresponding volume of medium.

The single most important advance in the use of centrifugal force to separate biologically important substances was the combination of mechanics, optics, and mathematics. Rotors for a centrifuge are either fixed angles, swinging buckets, continuous flow, or zonal, depending upon whether the sample is held at a given angle to the rotation plane, allowed to swing out on a pivot and into the plane of rotation, designed with inlet and outlet ports for separation of large volumes, or a combination of these.

Fixed angles generally work faster; substances precipitate faster in a given rotational environment, or they have an increased relative centrifugal force for a given rotor speed and radius. These rotors are the workhorse elements of a cell laboratory, and the most common is a rotor holding 8 centrifuge tubes at an angle of 34°C from the vertical.

In a centrifugal field, the gravitational acceleration (g) is replaced by the centrifugal force. Ultracentrifugation is carried out at speed faster than 20,000 rpm.

Super speed ultracentrifugation is at speeds between 10,000 and 20,000 rpm. Low-speed centrifugation is at speeds below 10,000 rpm.

Working: Cooling Centrifuge

- Switch on the mains. The power indicator will get illuminated.
- Setting the Program Number: Press the PROG button. The required program number can be set by using the UP or DOWN.
- Setting the timer: Press the PROG button again. The display panel will show Zero and the timer setting is initialized. The required time setting can be made by pressing the UP arrow for the increment and DOWN arrow for decreasing the time. Set the time required for the program and release the button.
- Setting the temperature: Press the PROG again. Now the system is ready for temperature setting. Set the required temperature by pressing the UP or DOWN arrow till the required temperature is set. Release the button.
- Selection of RPM: Press the PROG button again. The option to select the display mode to read normal display in RPM\RCF can be selected by using the UP or DOWN
- Setting the rotor speed: Press the PROG button again. Use the UP or DOWN arrow to set the required speed. Release the button
- Setting the acceleration time: Press the PROG button again. Use UP or DOWN arrow to set the acceleration time within the range of 60s-240s. Release the button.
- Setting the deceleration time: Press the PROG button again. The system is ready to set the deceleration time. Use UP or DOWN arrow to set the deceleration time within the range of 45s– 240s. Release the button.
- Rotor Selection: Press the PROG button again. The system is ready to set the rotor Selection. The Rotor heads are numbered and select the specific rotor head number which you plan to use in this program. Use arrow UP or DOWN select the specific number of the Rotor head you plan to use. Release the button, saving the setting and locking the program.
- Now you have completed the setting of the parameters for the first program and the same is automatically registered and saved as parameters of the set program number as program 1. Press the RETURN key to come back to Normal Display mode.

Microfuge

Working: Microfuge

- Choose a flat area on your table
- Put the line chord into a suitable 220v 50Hz power point
- Timer can be set in pulse mode. Timed cycles from 1-15 minutes in

increment of 1 minute and continuous mode

- The RESET—OFF key is to be pressed whenever time or rpm setting is to be changed.
- An audible beep sounds at the beginning and on completion of the cycle

Points to Remember:

1. After centrifugation wipe the inner chamber and keep open to be dried.
2. Clean the rotor after use. There are chances for any spill of liquids used.
3. Notice if any voltage fluctuation occurs.
4. It shows drive fault if any error occurs. In that case switch off and then enter the program.
5. Always make sure the required temperature is attained before the rotor starts.

Magnetic Stirrer with Hot Plate

9. Magnetic Stirrer with Hot Plate

General Description: It is used to dissolve certain slowly dissolving chemicals and for mixing the solution. It is used in many biological labs.

Principle & Working: A magnetic stirrer is a laboratory device consisting of either a rotating magnet or stationary electromagnets creating a rotating magnetic field. This device is used to cause a stir bar immersed in a liquid to spin very quickly, agitating or mixing the liquid. A magnetic stirrer often includes a provision for heating the liquid. They are preferred over gear-driven motorized stirrers because they are quieter, more efficient, and have no moving external parts to break or wear out (other than the simple bar magnet itself).

Due to its small size, a stirring bar is more easily cleaned and sterilized than other stirring devices. Magnetic stirrers avoid two major problems with motorized stirrers. Firstly, motorized stirrers use lubricants, which can contaminate the reaction vessel and the product. Secondly, in motorized stirrers, the sealing of the connection between the rotating shaft of the stirrer and the vessel can be problematic, especially if a closed system is needed.

Magnetic stirrers also have drawbacks. For example, the limited size of the stirring bar means it can only be used for relatively small (under 4 liters) experiments. In addition, viscous liquids or thick suspensions are extremely difficult to mix using this method, although there are some stirrers with special magnets to overcome this problem.

10. UV-Visible Spectrophotometer

General Description: To measure the chemical constituents of the sample and to get spectrometric analysis of the given samples.

Principle: If a beam of light of intensity falls on a cell containing the sample, the emergent radiation intensity I is less intense than the incident radiation, since a portion of it is absorbed by the sample. The absorption is different at different wavelengths and is characteristic of the sample. This characteristic quantity is called the absorbance and may be calculated from the Beer-Lambert principle. The Beer-Lambert law states that in a sample, each successive portion along the path of the incident radiation, containing an equal number of absorbing molecules absorbs an equal fraction of the radiation that traverses it.

A spectrophotometer measures the relative amounts of light energy passed through a substance that is absorbed or transmitted. We will use this instrument to determine how much light of (a) certain

wavelength(s) is absorbed by (or transmitted through) a solution. Transmittance (T) is the ratio of transmitted light to incident light. Absorbance (A) = – log T. Absorbance is usually the most useful measure, because there is a linear relationship between absorbance and concentration of a substance. This relationship is shown by the Beer-Lambert law: A = ebc; Where e=extinction coefficient (a proportionality constant that depends on the absorbing species), b = path length of the cuvette. Most standard cuvettes have a 1-cm path and, thus, this can be ignored; c = concentration.

UV - Visible Spectrophotometer

Working:

1. Turn on the spectrophotometer and allow 10 minutes for the instrument to warm up before use.
2. Adjust the wavelength to that specified for the procedure you are using. Be sure the cover is closed on the cuvette holder and use the left knob on the front panel to adjust the dark current so that the meter is reading zero transmittance. At this point, you are simply adjusting the internal electronics of the instrument to blank out any residual currents. This adjusts the lower limit of measurements. It establishes that no light is equivalent to 0 transmittance or infinite absorbance.
4. Insert a clean cuvette containing the blank into the holder. Be sure that the tube is clean, free of fingerprints, and that the painted line marker on the tube is aligned with the mark on the tube holder. Close the top of the tube holder. The blank for this exercise is the solution containing no dopachrome, but all other chemicals. The amount of solution placed in the cuvette is not important, but is usually about 5 ml. It should approximately reach the bottom of the logo printed on the side of the cuvette.
5. Adjust the meter to read 100% transmittance, using the right knob on the front of the instrument. This adjusts the instrument to read the upper limit of the measurements and establishes that your blank will produce a reading of 100% transmittance (zero absorbance).
6. Remove the blank from the instrument and recheck that your 0 transmittance value has not changed. If it does, wait a few minutes for the instrument to stabilize and read steps 1–5. Periodically throughout the work, check that the calibration of the instrument is stable by reinserting the blank and checking that the 0 and 100% T values are maintained.
7. To read a sample, simply insert a cuvette holding your test solution and close the cover. Read the transmittance value directly on the scale.
8. Record the percent transmittance of your solution, remove the test tube cuvette, and continue to read and record any other solutions you may have.

It is possible to read the absorbance directly, but with an analog meter (as opposed to a digital readout), absorbance estimations are less accurate and more difficult than reading transmittance. Absorbance can be easily calculated from the transmittance value. Be sure that you note which value you measure.

Absorption Spectrum

In the use of the instrument for determination of concentration (Beer-Lambert Law), the wavelength was preset and left at a single value throughout the use of the instrument. This value is often given by the procedure being employed, but can be determined by an analysis of the absorption of a solution as the wavelength is varied.

A dual-beam spectrophotometer divides the light into two paths. One beam is used to pass through a blank, while the remaining beam passes through the sample. Thus, the machine can monitor the difference between the two as the wavelength is altered. It scans a blank and places the digitized information in its computer memory. It then rescans a sample and compares the information from the sample scan to the information obtained from the blank scan. Since the information is digitized (as opposed to an analog meter reading), manipulation of the data is possible. These instruments usually have direct ports for connection to personal computers, and often have built-in temperature controls as well.

This latter option would allow measurement of changes in absorption due to temperature changes (known as hyper chromicity). These, in turn, can be used to monitor viscosity changes, which are related to the degree of molecular polymerization with the sample.

11. Water Bath

Water Bath with Stirrer

General Description: A water bath is an instrument used in the laboratory for carrying out biochemical, agglutination, inactivation and biomedical tests and even for industrial incubation procedures.

Principle: It is a system for the control of temperature in which a vessel containing the material to be heated is set into or over one containing water and receiving the heat directly.

Working: It has a double walled, outer body made of MS sheet, powder coated, inner body made of stainless steel, and 304 quality sheet glass wool insulation, fitted with 30ºC to 110ºC thermostat. By means of these, heat is transferred to the medium (water or oil) until reaching the temperature selected with a control device (thermostat or similar). It is provided with concentric rings with a diameter of 75 mm suitable to work on 220V AC supply. It is accompanied with a digital display temperature controller. In general they use water, but some baths use oil.

Before using the water bath, verify that it is clean and that accessories needed are installed. The steps normally followed are:

1. Fill the water bath with fluid to keep the temperature constant (water or oil). Verify that once the containers to be heated are placed, the fluid level is between 4 and 5 cm from the top of the tank.

2. Install the control instruments needed, such as thermometers and circulators. Use additional mounts provided for this purpose. Verify the position of the thermometer's bulb or thermal probe to ensure that the readings are correct.

3. If water is used as the warming fluid, verify that it is clean. Some manufacturers recommend adding products which prevent the formation of fungus or algae.

4. Put the main switch No. 1 in the ON position (the numbers identifying the controls herein correspond to those shown in the diagram). Some manufacturers have incorporated controls with microprocessors which initiate auto-verification routines once the ON switch is activated.

5. Select the operation temperature using the Menu No. 2 button and the buttons for adjusting the parameters.

6. Select the cut-off temperature (in water baths with this control). This is a safety control which cuts off the supply of electricity if it exceeds the selected temperature. This is selected also by using the menu button and is controlled by the parameter adjustment buttons.

7. Avoid using the water bath with the substances indicated below:

a) Bleach.
b) Liquids with high chlorine content.
c) Weak saline solutions such as sodium chloride, calcium chloride or chromium compounds.
d) Strong concentrations of any acid.

e) Strong concentrations of any salt.
f) Weak concentrations of hydrochloric, hydrobromic, hydro iodic, sulphuric or chromic acids.
g) Deionized water, as it causes corrosion and perforation in the stainless steel.

Safety

1. Avoid use of the water bath in environments where there are flammable and combustible materials. The equipment has components (resistors generating very high temperatures) which could start an accidental fire or explosion.

2. Always connect the equipment to an electrical outlet with a ground pole to protect the user and the equipment from electrical discharges

3. Use the water bath exclusively with non-corrosive or non-flammable liquids.

4. When working with substances that generate vapours, place the water bath under a chemical hood or in a well-ventilated area.

5. Remember that liquids incubated in the water bath tank can produce burns if hands are inadvertently placed inside it.

6. Take into account that the water bath is designed for use with a liquid inside the tank. If the inside is dry, the temperature of the tank can become very high. Use the diffusing tray for placing the container inside of the filled tank of the water bath. This has been designed for distributing the temperature in a uniform way.

8. Avoid using the water bath if any of its controls is not working, e.g. the temperature or limit controls.

Cleaning

Frequency: Monthly

1. Turn off and disconnect the equipment. Wait until it cools to avoid the risk of burns and accidents.

2. Remove the fluid used for heating. If it is water, it can be poured through a siphon. If it is oil; collect into a container with an adequate capacity.

3. Remove the thermal diffusion grid located at the bottom of the tank.

4. Disassemble the circulator and clean to remove scale and potential algae present.

5. Clean the interior of the tank with a mild detergent. If there is any indication of corrosion, use substances for cleaning stainless steel. Rub lightly with synthetic sponges or equivalent. Avoid using steel wool to remove rust stains as these leave particles of steel which could accelerate corrosion.

6. Avoid bending or striking the temperature control capillary tube generally located at the bottom of the tank.

7. Clean the exterior and interior of the water bath with clean water.

12. Deep Freezer

Principle & Working: The basic principle behind a freezer is evaporation. When a liquid evaporates it causes the surrounding area to cool. Water can't be used in freezer though, because it evaporates at too

high a temperature. But some liquids evaporate at very low temperatures. For example, Isobutane (becoming more common in domestic freezers) evaporates at very low temperatures. This ability to evaporate at very low temperatures means that it cools surfaces which are already very cold.

Evaporation is affected by air pressure. The higher the air pressure, the lesser a liquid will evaporate.

1. The Compressor takes in the refrigerant (as gas); raise the air pressure which converts the refrigerant gas to liquid.
2. As the refrigerant liquid flows from the Compressor to the Expansion Valve the high air pressure stops it evaporating and instead it gives off heat and becomes cooler.
3. The refrigerant liquid flows through the expansion valve where the air pressure is much lower. This causes the refrigerant liquid to evaporate which causes the pipe to become very cold inside the freezer.
4. One key component of the freezer is the thermostat. The thermostat senses the temperature inside the freezer and when it drops below a certain temperature it turns off the motor so the flow of the refrigerant liquid stops. When the temperature rises above a certain level the thermostat turns on the motor and the refrigeration process restarts.

Refrigerant Liquids

Different freezers have different refrigerant liquids. Which liquid is used is important for two main reasons. Firstly different liquids are more or less efficient for use in a freezer. Less efficient liquids will use more electricity and therefore cost you more to run your freezer.

Secondly, some refrigerant liquids contribute significantly more than others to global warming and destruction of the ozone layer. Pre-1990 many freezers used CFC (Chloro Flouro Carbon) which caused significant environmental damage to the atmosphere. This was then replaced with MFC which does not destroy the ozone layer but still does contribute to global warming. Currently HC (Hydro Carbon) and Isobutane are used as refrigerant liquids in most domestic freezers. Both are good with Isobutane being the best.

13. Muffle furnace

A muffle furnace is a front-loading box-type oven or kiln for high-temperature applications such as fusing glass, creating enamel coatings, ceramics and soldering and brazing articles. They are used in order to determine what proportion of a sample is non-combustible and non-volatile that is called as ash.

Principle & Working: The furnace chamber is heated by electric resistance elements and is insulated with ceramic fiber insulation. The controller is located under the furnace chamber and is well insulated from the heat generated in the furnace chamber. A door safety switch removes power to the heating elements whenever the furnace door is opened. The temperature is controlled by one of three types of controllers.

Muffle furnace

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Chapter 4

Protocol for the preparation of Reagents

Buffers

A buffer, as defined by Van Slyke is "a substance which by its presence in solution increases the amount of acid or alkali that must be added to cause unit change in pH". Buffers are thus very important components in experiments designed to study biological reactions by maintaining a constant concentration of hydrogen ions within the physiological range.

Measurement of pH

pH can be measured in many ways. An accurate and practical method of measuring pH involves the use of pH meter. It consists of a glass electrode with a glass bulb made of very thin glass that is permeable to hydrogen ions. Standardization against a buffer of known H^+ concentration is required since concentration of H^+ inside the bulb of glass electrode changes with time.

The pH scale runs from 0 to 14, with 7 representing neutrality and pH value above 7 characterize basic conditions and pH value below 7 represent acidic conditions.

Preparation and dilution of solutions

1. Percent solution

A solution in which the exact concentration of the solute in 100 ml of a liquid is known as percentage solution. The concentration may be expressed as weight or volume

Percentage (v/v) = x ml in 100 ml of solution

Percentage (w/v) = x g in 100 ml of solution

Preparation of concentrated solutions

1. Molar solution (M) (mole): The SI unit of amount of substances, equal to the quantity containing as many elementary units as there are atoms in 0.012kg of Carbon-12 or One gram molecular weight of the solute dissolved in 1000 ml of solvent.
2. Normal solution (N): One gram equivalent weight of the solute dissolved in 1000 ml of distilled water.
3. Molal (mol): containing one mole of solute per kilogram of solvent.

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Chapter 5

Proximate analysis of food samples

Proximate analysis of a food sample determines the total protein, fat, carbohydrate, ash, and moisture reported as the percentage composition of the product. There are food composition tables that contain proximate analyses for a large number of established foods, and as new food items are added to our shopping baskets their proximate compositions are added to the database, periodically, in supplements.

Total Protein

The total protein content of a food sample is estimated as total nitrogen (*Kjeldahl method*) after digestion, salt neutralization and titration of the ammonia released against standard acid.

Total Carbohydrate

There are disputes about what should be included in the calculation of carbohydrate content. The chief difference lies in the reporting of "total" carbohydrates, made up of monosaccharaides (sugars) and polysaccharides (starch and cellulose, including soluble and insoluble fiber). Some analysts report fiber separately and others include it along with the available sugars and starches to give total carbohydrate. Current qualitative and quantitative interest in fiber for nutritional marketing and food labeling requires a separate figure to be available anyway. A further anomaly lies in the practice of reporting total carbohydrate as the difference, after summing the quantities of the other components. The analytical implications of these uncertainties are discussed in relation to extraction methods.

Total Lipid (Fat)

Total lipid (fat) content may be calculated simply as the material extracted into diethyl ether. However, there are concerns over the availability of the many chemically different forms of fat and at least a digestion of the protein and carbohydrate would ensure the efficient release of fat from the tissue. Modern solvent-extraction methods are employed to improve the reproducibility, but there are still discussions about the nature of the lipids extracted under the different conditions.

Moisture Content

Moisture is the major component of food. The moisture content of any food is determined not only to analyze the chemical composition of food material but also to assess the shelf-life of the product. Moisture assays can be one of the most important analyses performed on a food product and yet one of the most difficult from which to obtain accurate and precise data. The most important method of moisture analysis is oven drying method.

In oven drying methods, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample. The amount of moisture determined is highly dependent on the type of oven used, conditions within the oven, and the time and temperature of drying. Various oven methods are approved by AOAC (Association of Official

Agricultural Chemists) International for determining the amount of moisture in many food products. The time required may be from a few minutes to over 24 h.

Total Solids (Ash)

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in food stuff. Total solids or ash is recorded as the material remaining after the removal of all vaporizable material by high temperature combustion in a furnace (at 500ºC).

Dry ashing refers to the use of a muffle furnace capable of maintaining temperatures of 500–600^{o}C. Water and volatiles are vaporized, and organic substances are burned in the presence of oxygen in air to CO_2 and oxides of N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides, and silicates. Elements such as Fe, Se, Pb, and Hg may partially volatilize with this procedure, so other methods must be used if ashing is a preliminary step for specific elemental analysis.

Wet ashing is a procedure for oxidizing organic substances by using acids and oxidizing agents or their combinations. Minerals are solubilized without volatilization. Wet ashing often is preferable to dry ashing as a preparation for specific elemental analysis. Wet ashing often uses a combination of acids and requires a special per chloric acid hood if that acid is used.

Crude fiber

It is a measure of the quantity of indigestible cellulose, pentosans, lignin, and other components of this type present in foods. It is the residue of plant materials remaining after solvent extraction followed by digestion with dilute acid and alkali. These components have little food value but provide the bulk necessary for proper peristaltic action in the intestinal tract.

The determination of crude fiber content of any food sample includes the sequential acid and alkali hydrolysis of the sample. The method is applicable to cereals and cereal products as well as to certain products containing less the 1 % of crude fiber. It consists in boiling with a mixture of acetic acid, nitric acid and trichloroacetic acid (after any necessary grinding and defatting). Then separation and washing of the insoluble residue on a filter crucible. Drying and weighing of the insoluble residue, and determination of the loss of mass on incineration in a muffle furnace.

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Experiment No.1: Determination of total protein of a food sample

The crude protein in samples was determined using **Kjeltec distillation unit**.

Procedure

- A finely grounded 0.8 g of food sample powder was transferred to a digestion tube
- Add 0.5 g of digestion mixture and 12 ml of concentrated H_2SO_4.
- The sample was digested in a digestion unit till it became colorless.
- Then the digestion tubes were cooled and transferred to the distillation unit.
- 30 ml of 40% NaOH solution was allowed into the tube.
- Liberated ammonium gas was absorbed in 4% boric acid solution containing mixed indicator.
- The pink color of the boric acid solution was turned into green and this was titrated against 0.01N HCl until the pink color was obtained.
- The crude protein in per cent was obtained by using the following formula.

$$\text{Protein (\%)} = \frac{\text{TV} \times 0.014 \times 100\,(ml) \times 0.01 \times 100 \times 6.25}{\text{Weight of the sample (g)} \times \text{Aliquot used for distillation (ml)}}$$

where,

TV = Titre Value

Experiment No.2: Determination of total carbohydrate of a food sample

The procedure for carbohydrates estimation was carried out as follows.

- Five grams of the sample was clarified with 25 ml of 80% ethanol.
- About 0.5 ml of the clarified solution was taken in a 100 ml volumetric flask and diluted to the 100 ml mark with distilled water.
- From the sample solution, one ml was taken in a test tube, then add one ml of 5% phenol solution and 5 ml of 96% concentrated H_2SO_4.
- The sample was cooled to room temperature by keeping in a water bath at 25 to 30°C for 20 min.
- The absorbance of the prepared samples was observed at 515 nm using a spectrophotometer.
- The concentration of carbohydrates in the sample was determined using standard graph.

$$\text{Carbohydrate (\%)} = \frac{\text{X}}{0.1} \times 100$$

where, x = concentration of glucose from standard graph

Experiment No.3: Determination of total fat of a food sample

The crude fat content of the samples were estimated by **Soxhlet extraction** method using SOCS – PLUS apparatus

- Sample of 2 g was weighed using electronic weighing balance and transferred to a thimble.
- The weight of the empty beaker was recorded and all the beakers were loaded into the system. Eighty ml of petroleum ether was poured into the beaker from the top and boiled for about 80-90 min at 80°C.
- After the completion of process time, the temperature was doubled to 160°C for 15–20 min to collect the petroleum ether.
- All the beakers were removed and placed in a desiccator for about 5 min. The final weight of the beaker was noted down.

$$\text{Crude fat } (\%) = \frac{W_4 - W_3}{W_2 - W_1} \times 100$$

where,

W_1 = Weight of empty thimble, (g) W_2 = Weight of thimble + sample, (g)

W_3 = Weight of empty flask, (g) W_4 = Weight of flask + fat, (g)

Experiment No.4: Determination of moisture content of a food sample

The moisture content of the sample was determined by following oven drying method.

- Five g of sample was kept in a pre-dried moisture box. The mass of the sample was recorded as W_m.
- The box was placed in the hot air oven maintained at 105°C for 3 h.
- After drying, the box was kept in the desiccator and then weighed. The mass of the dried sample was recorded as W_d.
- All measurements were replicated thrice and the average moisture content was calculated as,

$$\text{Moisture content } (m.c.\%) = \frac{W_m}{W_m + W_d} \times 100$$

where,

$m.c.$ = moisture content, percent wet basis

W_m = mass of water evaporated, g

W_d = mass of dry matter, g

Experiment No.5: Determination of ash content of a food sample

The total ash content of the sample was determined using muffle furnace

- 5 g of the sample was accurately weighed into a crucible (which was previously heated to about 600°C and then cooled).
- The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3-5 h at 600°C.
- It was then cooled in desiccator and weighed.
- The percentage of ash was calculated by using the following expression.

$$\text{Total ash}\,(\%) = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Experiment No.6: Determination of fiber content of a food sample

The crude fiber content in sample was determined by sequential acid and alkali hydrolysis method using **Fibra-Plus apparatus**.

- Food sample of 2 g was taken in a crucible (W).
- The sample was boiled in 1.25% sulphuric acid, subsequently boiled in 1.25% sodium hydroxide solution.
- The sample was dried in hot air oven at 100ºC till all the moisture was evaporated.
- The weight of the crucible before ashing was noted down (W_1).
- The obtained dried sample was ashed in a muffle furnace at 550ºC for 4 h.
- After ashing, the crucibles were cooled in a desiccator and weighed (W_2).
- The residue obtained after subtraction of the ash was regarded as fiber.
- The crude fiber was obtained by using the following equation.

$$Crude\ fibre\,(\%) = \frac{W_1 - W_2}{W} \times 100$$

where,

W_1 = Weight of the sample before ashing, g
W_2 = Weight of the sample after ashing, g
W = Weight of the sample, g

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Chapter 6

Determination of chemical constituents of foods

Fruits and Vegetables

Fruits and vegetables are the source of many vitamins, minerals and other natural substances that may help protect you from chronic diseases. Eating fruits and vegetables of different colors gives your body a wide range of valuable nutrients, like fiber, folate, potassium, and vitamins A and C. During transport, the quality of various fruits and vegetables deteriorates. So, parameters responsible for deterioration of quality of fruits and vegetables should be determined:

- Titrable acidity
- Total soluble solids (TSS)
- Ascorbic acid
- Reducing Sugar
- Non Reducing Sugar
- Total Sugar
- Total phenolic compounds

Estimation of titrable acidity

The acid content of foods directly affects their flavour. Practically all foods contain an acid or a mixture of acids. These acids may occur naturally, may be produced by action of micro-organisms, or may be added in such products as ketchup or chilli sauce during their manufacture. Total acidity determinations are useful as a measure of this tartness. In some cases a high concentration of acid may be an indication of spoilage and rancidity while in other cases high acid content is necessary for preservation. Total acid is usually determined by titrating an aliquot of sample with a base of known strength using a suitable indicator to determine the end point. In the case of highly colored foods, such as tomatoes, accurate determination of the end point is very difficult when using an indicator; thus it is easier and more accurate to use electrometric methods when possible. The acidity is usually calculated and reported in terms of the predominant acid like citric acid.

Titrimetric method

Reagents

1. Sodium hydroxide: 0.1 N
2. Phenolphthalein indicator: Dissolve 1 g of indicator in 60 ml of 95 per cent ethyl alcohol and dilute to 100 ml with distilled water.

Preparation of sample

A. Juices: All fruit juices should be thoroughly mixed by shaking to ensure uniformity of sampling. Filter the juice through filter paper to remove the coarse particles which can result in an inaccurate measured sample.

B. Fresh fruits: In order to secure a sample of the water soluble acids, the fresh fruits must be crushed. This can be accomplished by placing the fruit in a small hand-operated press type juice extractor or a layer of cheese cloth and squeezing it tightly until the pulp is fairly dry. If the juice sample contains fibers and particles, the liquid should be centrifuged, or filtered until the juice is clear.

Procedure

- Weigh 25 g of macerated sample or pipette a 25 ml sample and transfer to a 250 ml volumetric flask.
- Dilute to 100 ml with distilled water. Shake and filter through Whatman No. 4 filter paper.
- Pipette 50 ml of the filtrate into a 250 ml Erlenmeyer flask and add 50 ml of distilled water.
- Add 0.3 ml of 1 % phenolphthalein solution and titrate with 0.1N sodium hydroxide solution until the pink color develops.
- Record the volume of the sodium hydroxide used and calculate the acidity.

Calculations

Total acid may be expressed on three different bases

A) As volume (ml.) of 0.1 N NaOH per 100 ml of sample:

Let x ml of 0.1N NaOH is used for 50 ml of the solution.

The amount of 0.1N NaOH used/100ml juice = x100= 2x

B) As grams of acid

To calculate results in terms of grams of acid, all acids present are calculated in terms of one acid. It is customary to express acids in oranges and other citrus fruits and tomato products as citric acid, in cider and other apple products as malic, in sauerkraut as lactic, in grape juice as tartaric acid and in vinegar as acetic. In general, the predominant acid present in the juice or product is used for calculation purposes using the 'theoretical' rather than the actual equivalent weight.

Calculate grams of acid in the aliquot titrated- gram acid in aliquot

$$\frac{\text{ml of 0.1 N NaOH x equivalent wt. of acid}}{10{,}000}$$

$$\frac{\text{ml 0.1 N NaOH used x factor*}}{\text{Volume of sample in ml}} \times 100$$

Estimation of mineral content

Minerals help the body to grow, develop and stay healthy and perform many different functions - from building strong bones to transmitting nerve impulses. Some minerals are also used to make hormones or maintain a normal heart-beat. Minerals are also important components of various enzymes, hemoglobin, chlorophyll in plants and electron transport system.

Reagents

1. Di acid mixture – Mix nitric acid and perchloric acid in 4:1 ratio just before use.
2. Hydrochloric acid (1%) – Add 1 ml of concentrated HCl in 50 ml distilled water and make up the volume to 100 ml with distilled water.

Procedure

1. Digest one gram powdered sample with 15 ml of diacid mixture ($4HNO_3$: 1 $HClO_4$) in a conical flask by heating on hot plate in open space till clear white precipitates settle down at the bottom of the conical flask.
2. Dissolve the precipitates in 1% HCl prepared in double glass distilled water, filter and make volume of the filtrate to 50 ml with double glass distilled water.

A. Micronutrients

The contents of iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) may be estimated, from the extract prepared, by Atomic Absorption Spectrophotometer.

B. Macronutrients

The amount of phosphorus, potassium and sodium may be estimated from the same extract by the procedures mentioned below:

I. Nitrogen

Total nitrogen in samples may be estimated by conventional Micro-Kjeldahl's method. The digestion should be done separately by sulphuric acid as described in experiment no. 3.

II. Phosphorus

Total phosphorus may be estimated calorimetrically by the method of Chen et al. (1956).

Reagents

1. Ascorbic acid (10%): Prepare by dissolving 10 g ascorbic acid in 100 ml of distilled water.
2. Ammonium molybdate 2.5%: Prepare by dissolving 2.5 g ammonium molybdate in 100 ml of distilled water.
3. Reagent C: Prepare by mixing 6N sulphuric acid, distilled water, 2.5% ammonium molybdate and 10% ascorbic acid in the ratio of 1:2:1:1 (v/v) at the time of use.

Procedure

1. Take one ml of the diluted extract (1 ml extract + 9 ml distilled water), prepared for determination of mineral composition, in a test tube and make volume to 4 ml with distilled water.
2. Then, add 4 ml of reagent C to it and mix well.
3. Incubate the contents at 37°C in a water bath for 90 min and cool at room temperature.
4. Read the absorbance at 820 nm against a suitable blank and calculate the phosphorus content with the help of a calibration curve of monopotassium dihydrogen orthophosphate and express as mg g^{-1} dry weight.

Another method for estimation of phosphorus is proposed by Fiske and Subbarow (1925) it is very sensitive method.

Reagents

1. Ammonium molybdate 2.5%: Prepare by dissolving 2.5 g ammonium molybdate in 100 ml of distilled water.
2. Sulphuric acid (10N): Prepare by mixing 278 ml concentrated sulphuric acid in 500ml, chilled distilled water, cool and make volume to 1000ml.
3. Solution SS: Prepare by dissolving 29g sodium bisulphite and one g sodium sulphite in 200ml distilled water.
4. 1,2,4 amino naphthol sulphonic acid reagent: Weigh 0.5 g 1,2,4 amino naphthol sulphonic acid, transfer it to mortar, add 10ml SS solution, grind with pestle and make volume to 200ml with SS solution. Filter through whatman no. 1 filter paper. Store the reagent in dark.
5. Standard phosphorus solution: Dissolve 1.099g KH_2PO_4 in 250ml distilled water. This will be a standard stock solution of phosphorus having 1mg phosphorus/ml. Prepare 10, 20, 40, 60, 80 and 100 µg/ml solution by diluting standard stock solution.

Procedure

1. Take two ml of the diluted extract (1 ml extract + 9 ml distilled water), prepared for determination of mineral composition, in a test tube.
2. Add 0.2 ml of 10N H_2SO_4 to it and mix well.
3. Add 0.4 ml 2.5% ammonium molybdate solution, shake and add 2.2 ml distilled water followed by 0.2 ml amino naphthol sulphonic acid reagent, again shake the test tubes.
4. Incubate the contents at room temperature for 10minutes for color development.
5. Read the absorbance at 660 nm against a suitable blank and calculate the phosphorus content with the help of a calibration curve of monopotassium dihydrogen orthophosphate and express as mg g^{-1} dry weight.

III. Potassium and Sodium

Potassium (K) and sodium (Na) content in the digested sample extracts can be estimated by digital flame photometer after calibrating with standard potassium and standard sodium solutions, respectively.

Determination of soluble protein content by Lowry method

The method is based on the principle that the phenolic group of tyrosine and trytophan residues (amino acid) in a protein produce a blue purple colour complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau reagent which consists of sodium tungstate, molybdate and phosphate. The method is sensitive down to about 10 µg/ml and is probably the most widely used protein assay.

Reagents

1. Reagent A - 2% Na_2CO_3 in 0.1 N NaOH
2. Reagent B - 1% solution of $CuSO_4$ $5H_2O$ in distilled water
3. Reagent C - 2% sodium potassium tartarate
4. Reagent D - Prepare fresh before use by mixing reagents B and C in 1:1 ratio
5. Reagent E - Prepare by adding 1 ml of reagent D to 50 ml of reagent A
6. Reagent F - 1 N Folin-Ciocalteau reagent (Mix Folin-Ciocalteau reagent 2N and distilled water in 1:1 ratio)

7. Reagent G - 20% Trichloroacetic acid

Procedure

1. Precipitate soluble proteins in the enzyme extract by 20% TCA by keeping 1ml enzyme extract and equal amount of 20% TCA in centrifuge tubes for 1h. Then, centrifuge at 10,000 rpm for 20 minutes. Remove supernatant and redissolve residue in 5 ml of 0.1N NaOH.
2. To 1 ml of suitably diluted protein sample add 5 ml of reagent E, mix well and keep at room temperature for 10 min.
3. Then add 0.5 ml of reagent F and mix the content immediately on a vortex mixer. Read the color intensity at 660 nm after 30 min.
4. Calculate the equivalent amount of protein from a standard curve prepared by using bovine serum albumin (20-200 µg ml^{-1}).

Estimation of phenolic compounds

Phenols, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. They include an array of compounds like tannin, flavanols etc. and occur in all parts of the plants where they offer resistance to diseases and pests. Grains containing high amount of polyphenols are resistant to bird attack. Estimate the phenolic compounds using the same extract prepared for soluble sugar estimation.

Estimation of total phenols

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue). Total phenols may be estimated by the method of Swain and Hillis (1959).

Reagents

1. Folin – Ciocalteau reagent: Dilute the Folin-ciocalteau reagent (2N) with distilled water in 1:1 (v/v) ratio before use.
2. Saturated sodium carbonate solution: Dissolve the anhydrous sodium carbonate (35.0 g) in 100 ml of distilled water by heating on a water bath at 70-80°C. Cool the contents for overnight and use the supernatant.

Procedure

1. Take one ml of the extract in a test tube and dilute with distilled water (7.5ml).
2. Mix well the contents. Add to this, 0.5 ml of diluted Folin - Ciocalteau reagent.
3. Shake the tubes thoroughly and after 3 minutes, add 1 ml of saturated sodium carbonate and make total volume to 10 ml with distilled water.
4. Allow the tubes to stand for 1 h and read the absorbance at 725 nm using spectrophotometer.
5. Use distilled water in place of sample extract as reagent blank and rest of procedure for blank is same.
6. Determine the amount of total phenols in the sample from the standard curve prepared simultaneously by taking tannic acid as the standard phenol and express the data as mg g^{-1} dry weight.

Determination of peroxide value

Peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats. It is a measure of the concentration of peroxides and hydro peroxides formed in the initial stages of lipid oxidation. Milli equivalents of peroxide per kg of fat are measured by titration with iodide ions. High peroxide values are a definite indication of a rancid fat. Peroxide value is determined by the standard method of AOAC (1990).

Reagents

1. Glacial acetic acid
2. Saturated potassium iodide solution: Prepare the solution by dissolving excess of KI in distilled water. Use freshly boiled and cooled water. Store the solution in a dark bottle.
3. Sodium thiosulphate solution (0.01N): Dissolve 0.248 g of sodium thiosulphate (Molecular weight 248.12) in small quantity of water and dilute it to 100 ml with water.
4. Starch solution (1%): Dissolve 100 mg soluble starch in cold distilled water to make thin paste. Add boiling distilled water to this paste and further boil the solution for one minute while stirring. When completely dissolved, make the volume to 10 ml.

Procedure

1. Take 100 mg fat in a conical flask and dissolve in 10 ml glacial acetic acid.
2. Add 1 ml of saturated potassium iodide solution to the mixture and keep for 2 minutes with occasional shaking.
3. Add 20 ml of distilled water and 1 ml of starch solution.
4. Titrate the contents immediately with 0.01N $Na_2S_2O_3$ till the end point.
5. Calculate the peroxide value (PV) using the following formula:

$$\text{PV (meq. peroxide/kg of fat)} = \frac{S \times N}{W}$$

Where,

S = ml of $Na_2S_2O_3$ used (Test value– Blank value)

N = Normality of $Na_2S_2O_3$

W = Weight of fat in gram

Ascorbic acid or Vitamin C

Vitamin C or Ascorbic acid is an enediol isomer of 2-keto-l-glucanolactone with a configuration similar to that of L-glucose. Oxidation of ascorbic acid gives rise to dehydro-ascorbic acid and both forms are physiologically active.

Principle:

Titrimetric estimation of Vitamin C is conventionally done using 2,6-dichlorophenol indophenol dye solution. This dye is blue in alkaline solution and red in acidic solution. Ascorbic acid reduces the dye to a colorless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0 -3.5.

Reagents

(a). 4% Oxalic acid: 40 g of oxalic acid is dissolved in 100ml of distilled water (w/v)

(b). DCPIP dye solution: Dissolve 0.250 g of sodium salt of 2,6-dichlorophenol indophenols in about 500 ml of water containing 0.210 g of $NaHCO_3$ and dilute to 1 liter of water. Store the

solution in refrigerator and standardize it with freshly prepared standard solution of ascorbic acid every time just before use.

(c). Standard ascorbic acid ($C_6H_8O_6$): 0.01% Ascorbic Acid dissolved in oxalic acid.

Procedure:

1. Take 5 g of fruit sample (filtered juice) make up to 100 ml with 4% oxalic acid.
2. Take 5 ml of the sample from the 100 ml and add 10 ml 4% oxalic acid and titrate against the dye 2,6-dichlorophenol indophenol
3. The end point is determined by the appearance of pink color which should persist for at least 15 seconds.
4. Standardization of the dye solution: The dye solution is needed to be standardized simultaneously. From this 5 ml of standard ascorbic acid solution is taken and to this, 10 ml of 4% oxalic acid is added. Mixed well and titrated against DCPIP.

Results

Titer value (TV) is noted and this will be in a range between 0.1 – 1.0 ml

Calculation

0.5 mg/V_1 ml (3.3) x V_2(T.V)/5 ml/Wt. of sample (5 g) x 100

60.6 x T.V =……mg/100g.

Total Titratable Acidity

Acids are important constituents in fruits as together with sugars, they determine quality and taste of the fruits. Maturity of many fruits for their harvest is also judged from their level of acids along with sugars or the soluble solids. Fruits contain organic acids and among inorganic acids only phosphoric acid is present. Acids that are accumulated in fruits are largely synthesized in leaves and are translocated to fruits.

Principle:

The total acidity of the fruit could be determined by titrating a known amount of aqueous extract of it against an alkali solution of known normality. It is expressed as equivalence of any organic acid, eg. Citric, Malic etc.

Reagents:

(a) Sodium hydroxide solution: Make 50 ml 0.1N NaOH by dissolving 0.2 g of NaOH in 50 ml water

(b) Phenolphthalein indicator: Approx. 0.5% in 80 % ethanol

Procedure

1. Take 5 ml of sample and add 20 ml water and heat for 10 minutes.
2. Make up to 50 ml with water
3. Take 10 ml from the sample
4. Few drops of phenolphthalein solution is added and shaken well.
5. A burette is filled with 0.1 Normal Sodium hydroxide solution after washing and rinsing
6. Titration is done and the end-point is determined by the appearance of pink color and its persistence for at least few seconds.

Results

Titer value ranges from 0.1-1.5 ml

Calculation:

1 ml of 0.1N NaOH solution can neutralize 0.064 g of citric acid. Therefore, percentage of total titratable acidity in the sample as equivalence of citric acid is,

V_1x (T.V) x N (0.1) x 0.064 x 250 (50)/50 x 100/W (5)

0.128 x T.V=……..%

Reducing Sugar

In fruits both reducing and non-reducing sugars are present in varying amount. Reducing sugars are those hexose sugars, which can reduce compounds such as alkaline silver nitrate solution, cupric salt solution etc., because they themselves are oxidized.

Non reducing sugar (sucrose) is a disaccharide and cannot reduce alkaline silver nitrate or cupric acid solution.

Principle:

When sugars are extracted and titrated, the reducing sugars only take part in the reaction in making reduction, but the non-reducing sugars that are present in it, do not take part in reduction and remains as such. Accordingly only the reducing sugars are estimated by titration.

Reagents:

a) Fehling's solution- 5 ml Fehling's A+5 ml Fehling's B + 20 ml water
b) 45% Lead Acetate: 45 g of Lead Acetate in 100 ml water
c) 22% Oxalic Acid: 22 g of Oxalic Acid in 100 ml water

Procedure:

1. Take 25 g of sample (filtered juice) and heat for 3 minutes, till it turns a curd like appearance.
2. Add 2 ml of 45% lead acetate and wait for two minutes
3. Add 22 % oxalic acid to the sample to remove excess Lead acetate.
4. Wait till a yellowish tint appears and add NaOH until the bubble retains in the sample to neutralize the solution.
5. Make up to 250 ml and titrate against hot Fehling's solution. Add Methylene Blue at the end point and heat.
6. End point of the reaction is green color appearance. On addition of methylene blue and heating red color appears.

Results

Titer value range is 8-11 ml.

Calculation

0.05 x 250/V (T.V) x 100/w (25)

50/T.V =……g of glucose/100 g of juice

Total sugar

Principle

The non-reducing sugars which are not titratable are first hydrolyzed to reducing sugars. Thus after hydrolysis, the non-reducing sugars are converted to reducing sugars while the reducing sugars that are already present in the sample remain unchanged. Accordingly, all the

sugars those are present after hydrolysis remain as reducing sugars. This is conveniently termed as total sugars.

Reagents

a) Fehling's solution- 5 ml Fehling's A+5 ml Fehling's B + 20 ml water
b) 45% Lead Acetate: 45 g of Lead Acetate in 100 ml water
c) 22% Oxalic Acid: 22 g of Oxalic Acid in 100 ml water

Procedure

1. Procedure is same for reducing sugar, the volume is made up to 250 ml.
2. Out of the 250 ml sample solution, take 50 ml and add 5 g citric acid
3. Heat the sample and make up to 250 ml with water.
4. Titrate against the Fehling's solution.
5. End point of the reaction is a green color appearance.
   On addition of methylene blue and heating brick red color appears.

Result

Titer value range is 7-9ml

Calculation

0.05 x 250/ T.V x 250/50 x 100/W (25)

Non Reducing Sugar

The non-reducing sugars present in the sample may be determined from the values of the total and reducing sugars as follows

Percentage of non-reducing sugar = (Percentage of total sugar – reducing sugar)* 0.95

Total Soluble Solids

Total soluble solids (TSS) of a given sample of fruit juice representing various chemical substances present in it in soluble form. It indicates a measure of sugars present in the sample. The amount of TSS present in the juice of a fruit is also considered to be a reliable index in judging its maturity. In accordance with the harvest – maturity of many fruits is assessed in considering the TSS of their juices.

Principle

The TSS of a given fruit juice sample is determined in a quicker way with the help of the refractometer. The instrument works on the principle of refractive index of the sample and gives the refractive index as ˚Brix.

Requirements

A hand Refractometer, a dropper or a glass rod, blotting paper, absorbent cotton, rectified sprit, distilled water.

Procedure

- The lid that is covering plate of the refractometer which rest over the prism-plate and is attached with it at the base end with a hinge which can be unfolded backward. By doing so, both the lid and the prism-plate are exposed.
- The lid and the prism-plate are then carefully washed with a jet of clean water to ensure that they have no stain on its surface.

- Water adhered on the prism plate and the lid as well as the surrounding parts of them, if any is completely wiped off with blotting paper or absorbant cotton.
- The lid of the prism plate is then washed with distilled water and the water adhered on them is blotted out. The cleaning is best done by rubbing the lid and the prism plate gently and carefully with absorbant cotton, soaked with rectified spirit.
- Then, with the help of a previously cleaned dropper or a glass rod, a drop of distilled water is carefully dropped on the surface of the prism plate. The lid is folded forward and placed over the prism plate to cover it. At this position, the lid and the prism plate are firmly held together with fingers to avoid unfolding of the lid.
- The refractometer is held to point towards light. The eyepiece of it is brought close to any eye of the observer who should look into the eye-piece to have a view of the image of the scale. The scale focusing knob should be conveniently rotated to adjust it at such a position where the scale is most clearly visible. The shaded part would be seen to insert the unshaded part at zero position of the scale which indicates no reading with respect to distilled water. If it is not there then the reading should be brought to zero by rotating the scale calibrating screw.
- The lid is then folded backward. The distilled water that remains adhered over the lid and the prism-plate is completely blotted out and dried in air for a few minutes.
- A clear sample of fruit juice, TSS of which is to be determined is taken in the dropper, or a drop may be taken with the glass rod. A drop of juice is then carefully placed on the prism plate.
- Reading of the juice sample as ^{o}Brix is obtained and amount of TSS is expressed accordingly.

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Chapter 7

Microbial Analysis of foods

Microbiological Standards

Found in the Food Regulations Act 1984.

Cheese

The cheese shall contain not more than:

100 *Escherichia coli* per gram.

100 *Staphylococcus aureus* per gram.

A 50 g sample shall be free from Salmonella.

Ice cream, frozen confections

When subjected to the test described in the Fourth Schedule to the Food Regulations Act 1984, 3 out of 5 replicate portions of 0.1 millilitre shall not give evidence of acid formation and gas formation as described in that schedule. Tubes showing evidence of acid and gas formation shall be subcultured to an additional tube of test media and incubated according to the test prescribed in the Fourth Schedule as a confirmatory test.

Milk

(a) Raw milk, raw cream

Standard plate count at 30°C <150,000 colonies/ml.

(b) Pasteurized milk and pasteurized milk products

Includes - standardized milk, recombined milk, flavored milk, skim or non-fat milk, reduced-fat milk, cream, whipping cream or whipped cream, reduced cream or pouring cream, light cream, sour cream and recombined cream and any other milk product

Standard plate count at 35°C <50,000 colonies/ml.

(c) UHT (Ultra heat treated) milk and UHT milk products

UHT products shall be sterile when subjected to the test in the Sixth Schedule to the Food Regulations Act 1984.

Pulped egg

When subjected to the resazurin test described in the Seventh Schedule to the Food Regulations Act 1984 the reduction time shall be not less than 7 hours.

Yoghurt

pH <4.5.

Lactic acid producing bacteria ≥ 106 per ml

Food containers

The Food Hygiene Regulations Act 1974, contains requirements governing the "Microbiological Standards" for bottles, jars, or jugs, in that residual bacterial plate counts may not exceed:

(i) More than 1/millilitre of containing capacity, or

(ii) More than 1/square centimetre of surface area.

Table 1. The interpretation of Microbiological analysis

	Result category	Interpretation	Action
Pass	Good	Results are within expected microbiological levels for this type of product (lower range) and present no food safety concern.	None
	Acceptable	Results are within expected microbiological levels for this type of product (upper range) and present no food safety concern.	None
Fail	Unsatisfactory	Results are outside the expected microbiological levels for this type of product, present no food safety concern, but might indicate poor food handling practices.	Further samples are taken for testing. If these return good or acceptable results no action is taken. If these return unacceptable results the business is inspected to determine if food handling controls and hygiene practices are adequate. A product withdrawal may be considered while further testing occurs.
	Potentially hazardous	Results are outside of the expected microbiological levels for this type of product and present a potential food safety concern.	Inspect supplier to determine if food handling controls and hygiene practices are adequate; consider a product recall.

MICROBIOLOGICAL EXAMINATION OF MILK

Methylene blue reductase test

Principle:

This reductase test is based on the oxidation-reduction activities of the bacteria present in the milk sample. The indicator used in the reaction is methylene blue which is color sensitive to oxygen concentration. The indicator is blue in the oxidized state and leuco or white in the reduced conditions. The speed of color disappearance of methylene blue is proportional to the microbial load in the milk sample. The more the bacteria present the faster will be the reduction.

The classification of milk as per methylene blue reductase test is as follows.

1. Class I - Excellent, not decolorized in 8 hours.
2. Class II - Good, decolorized in less than 8 hours but not less than 6 hours.
3. Class III - Fair, decolorized in less than 6 hours but not less than 2 hours.
4. Class IV - Poor, decolorized in less than 2 hours.

Requirements:

Milk sample, methylene blue solution, Mc Cartney bottles, pipettes, water bath set at 37°C, distilled water, Bunsen burner.

Note

All the glass wares were sterilized before use.

Procedure

1. Methylene blue solution was prepared by dissolving 1 mg methylene blue powder aseptically in 25 ml of distilled water.
2. Transferred 10 ml of milk sample into sterile Mc Cartney bottle using sterile pippet.
3. Added 1 ml of methylene blue solution to the milk sample using a separate sterile pipette.
4. The bottle was closed with the stopper.
5. The contents of the tube were mixed by gently inverting it 2-3 times.
6. Incubate the Mc Cartney bottle in a water bath at 37°C for 6 hours.
7. Controlled tubes containing 10 ml boiled milk and 1 ml of methylene blue was also incubated.
8. Recorded the time for discoloration.

MICROBIAL ANALYSIS OF ICE CREAM AND SOFT DRINK

Principle

The bottled beverages including non-pasteurized non-carbonated soft drink should conform to the minimum requirement for microbiological criteria of the WHO standard. Only pasteurized milk was used in the manufacture of ice cream. Samples were received in the frozen state, melted one were rejected. The frozen samples were melted immediately before examination. The spread plates ensure an aerobic environment for microorganisms present in the food sample.

Requirements

Ice cream sample, soft drink sample, nutrient agar, pipettes, petriplates, test tubes, L rod, alcohol.

Procedure

a) Ice cream sample:

1. 1 g of ice cream sample was weighed and added to 10 ml sterilized distilled water blanks and was labeled as 10^{-1} dilution.

2. Mixed gently by inverting the test tubes for several times.
3. Prepared serial dilutions of ice cream sample by transferring 1 ml from first dilution to sterile distilled water and mixed well.
4. This test tube was labeled as 10^{-2}.
5. The procedure was repeated up to 7th test tube with respective dilution 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} using different sterile pipettes.
6. Discarded 1 ml of the sample from 10^{-7} dilution.
7. 0.1 ml of the diluted sample from particular dilution were pipetted out into nutrient agar plates and was spread uniformly using a sterilized L-rod.
8. Kept the plates in an upright position for few minutes.
9. Incubated the plates in an inverted position at 37°C for 24 hours.
10. Examined the plates for bacterial colonies.

b) ***Soft drink sample:***

1. 1 ml of soft drink was added to 9 ml sterilized distilled water blank and was labeled as 10^{-1} dilution.
2. Mix gently by inverting the test tubes for several times.
3. Prepared serial dilution of the soft drink sample by transferring 1 ml from first dilution to sterile distilled water blank and mixed well.
4. This test tube was labeled as 10^{-2} dilution.
5. The procedure was repeated up to 7th test tube with respective dilution 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} using different sterile pipettes.
6. Discarded 1 ml of the sample from 10^{-7} dilution.
7. 0.1 ml of the diluted sample from particular dilution were pipetted out into nutrient agar plates and was spread uniformly using a sterilized L-rod.
8. Kept the plates in an upright position for few minutes.
9. Incubated the plates in an inverted position at 37°C for 24 hours.
10. Examined the plates for bacterial colonies.

Observation

The number of colonies formed in each plates were counted for both ice cream and soft drink samples. Plates with fewer than 30 colonies were designated as “too few to count” (TFTC) and plates with more than 300 colonies as “too numerous to count” (TNTC).

MICROBIOLOGICAL ANALYSIS OF FRUITS AND VEGETABLES

Principle

Fruits and vegetables are readily susceptible to microbial decomposition, and hence considered as perishables. The total number of bacteria present in the sample of fruits and vegetables and other frozen food can be enumerated by traditional pour plate or spread plate technique. A sterile food blender is essential to obtain the microorganism in suspension.

Requirements

Nutrient agar, fruit sample, vegetable sample, petriplates, pipettes, L-rod, Homogenizer (food blender), alcohol.

Procedure

a) Fruit sample

1. Using aseptic technique, 1 g of fruit was weighed.
2. Weighed food sample was transferred into 10 ml sterile distilled water blank and was labeled as 10^{-1} dilution.
3. Mix gently by inverting the test tubes for several times.
4. Using a sterile pipette, 1 ml of sample was transferred from 10^{-1} dilution to sterile distilled water blank and was labeled as 10^{-2} dilution.
5. Mixed gently.
6. The procedure was repeated up to 7^{th} test tube with respective dilution 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} using different sterile pipettes.
7. Discarded 1 ml of the sample from 10^{-7} dilution.
8. 0.1 ml of the diluted sample from particular dilution were pipetted out into nutrient agar plates and was spread uniformly using a sterilized L-rod.
9. Kept the plates in an upright position for few minutes.
10. Incubated the plates in an inverted position at 37ºC for 24 hours.
11. Examined the plates for bacterial colonies.

b) Vegetable sample

1. Using aseptic technique, 1 g of vegetable was weighed.
2. Weighed sample was transferred into 10 ml sterile distilled water blank and was labeled as 10^{-1} dilution.
3. Mix gently by inverting the test tubes for several times.

4. Using a sterile pipette, 1 ml of sample was transferred from 10^{-1} dilution to sterile distilled water blank and was labeled as 10^{-2} dilution.

5. Mixed gently.

6. The procedure was repeated up to 7th test tube with respective dilution 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} using different sterile pipettes.

7. Discarded 1 ml of the sample from 10^{-7} dilution.

8. 0.1 ml of the diluted sample from particular dilution were pipetted out into nutrient agar plates and was spreaded uniformly using a sterilized L-rod.

9. Kept the plates in an upright position for few minutes.

10. Incubated the plates in an inverted position at 37^{o}C for 24 hours.

11. Examined the plates for bacterial colonies.

Observation

The number of colonies formed in each plate were counted for both fruit and vegetable samples. Plates with fewer than 30 colonies were designated as “too few to count” (TFTC) and plates with more than 300 colonies as “too numerous to count” (TNTC).

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Chapter 8

Good Manufacturing Practices for foods

Food quality control is necessary to ensure that food aid supplies are safe, of good quality and available in adequate amounts, in time, at affordable prices to ensure an acceptable nutritional and health status for all population groups. The main food quality systems are HACCP (Hazard Analysis and Critical Control Point), GMP (Good Manufacturing Practices), and Codex Alimentarius.

Good Manufacturing Practices (GMPs) as defined by the Food and Drug Administration are the minimum sanitary and processing requirements for food companies. GMPs are fairly broad and general and can be used to help guide the development of Standard Operating Procedures (SOPs) which are very specific. GMP contains ten principles that introduce employees to critical behaviours established by FDA and industry leaders to maintain good manufacturing practices in plants.

The GMP portion of the Food Quality and Safety Program (FQSP) represents generally accepted, broad-based guidelines, developed from scientifically based principles and current knowledge of food safety practices. The guide focusses on risk reduction, not risk elimination. Current technologies cannot eliminate all potential food safety hazards from product eaten in a raw form. This guide should be used to help assess food safety hazards within the context of the specific conditions (climatic, geographical, cultural, and economic) that apply to your own operation, and to implement appropriate and cost-effective risk reduction strategies.

Guidelines for Developing Good Manufacturing Practices (GMPs)

- Personnel - disease control, hygiene, clothing, training, etc.
- Plant and grounds - construction and design, product flow, drainage, etc.
- Sanitary operations - general maintenance, cleaning and sanitizing, pest control, etc.
- Sanitary facilities and controls - water supply, plumbing, sewage disposal, rubbish and offal disposal, etc.
- Freezers and coolers - monitored and maintained to ensure temperature control, recording devices, alarms, etc.
- Equipment maintenance and calibration - adequate frequency for thermometers, recording devices, compressed air equipment, etc.
- Recall program - It is recommended that all RTE facilities develop a recall program and that mock recalls should be conducted periodically to ensure that the program works as planned.

The following guidelines for developing Good Manufacturing Practices for RTE operations are recommended for voluntary consideration and use in developing plant-specific procedures. These GMPs are not designed to control specific hazards, but are intended to provide guidelines to help processors' produce safe and wholesome products.

Receiving Meat

Incoming meat should be evaluated to ensure that it meets the plant-established purchase specifications. Trucks, containers and carriers of raw materials should be evaluated upon receipt to ensure that the conditions meet plant requirements for transporting meat. All incoming meat should be coded/identified for plant use and for the in-plant tracking system.

Non-Meat Items

Producers of RTE products will need to make sure that all non-meat items, such as packaging materials, seasonings/spices, etc. meet the plant-established specifications. USDA currently requires companies to have a Letter Of Guarantee (LOG) from suppliers of non-meat ingredients relating to the use of food grade substances, foreign materials, pest control programs, etc. After the company accepts the non-meat items, then these items should be stored, handled and used in a manner that will maintain the integrity of the items.

Storage of Raw Materials

It is recommended that raw materials be used on a First-In/First-Out (FIFO) basis or according to a plant specified product rotation/inventory control schedule, such as the oldest borne date. Raw materials should be stored at temperatures that maintain proper product condition. Frozen materials should be kept frozen, unless tempering or thawing is required prior to use. The package/pallet integrity must be maintained throughout the storage period to maintain the condition of the material. Product identity in storage should allow for the in-plant tracking system.

Tempering/Thawing of Frozen Materials

If tempering or thawing is required prior to use, then it should be done in a time/temperature controlled manner, which is adequately monitored and documented. The product package integrity is important during this process. The product's traceability should be maintained throughout the tempering/thawing process.

Processing

Processing includes the application of the heat treatment, and it may include but is not limited to -weighing, mixing, blending, grinding, forming, stuffing, or other activities conducted prior to applying the heat treatment.

An organoleptic evaluation of the raw material ingredients should be completed prior to adding the meat to the batch. If applicable, the ingredients should be evaluated for chemical composition (% fat and lean) to formulate product to desired endpoint. Procedures for ensuring proper end product characteristics (i.e., weights, physical characteristics, quantity, etc.) should be in place.

The in-plant tracking mechanism should allow for batch identification and time of batch production, and may sublet the batch to a cooking lot if applicable. Establishments should have validated HACCP programs that include appropriate controls for identified hazards throughout the processing system.

Post-Processing Handling

It is very important that RTE producers recognize the importance of preventing cross contamination of post-processed (after applying the heat treatment) products with raw materials. All operations should have process control mechanisms to prevent cross contamination. The four factors outlined below can impact the establishment's control.

1. Facility Design:

The optimal facility design is to provide completely separate areas for raw and cooked processing. It is important that processing areas meet the "Clean Room Concept" including construction requirements provided by USDA, FDA and/or other organizations. The "Clean Room Concept" means that establishments should develop and maintain clean room standard operating procedures to eliminate cross contamination between RTE products and raw materials. This provides a mechanism for minimizing exposure of RTE products to microbial contamination.

Specific controls that may be addressed in the Clean Room Concept design/SOPs include:

- Physical barrier (preferably from floor to ceiling) for separating raw and cooked processing areas
- Employee traffic flow to prevent cross-over between raw and cooked areas
- Positive air flow in exposed product packaging rooms
- Use of foot baths before entrance into a RTE area, including preparation of sanitizing agent, schedule for changing, etc.
- Separate frocks, utensils, etc.
- Proper design, use and cleaning of drains
- Designated equipment and tools for RTE when possible

Due to plant design, complete separation is not always a realistic option. If a physical barrier cannot be added, then additional steps should be taken to help minimize the risk of contaminating post-processing products. For example, if an establishment has only one packaging room that must be used for both raw and RTE products then it should designate separate "processing times." The RTE products could be packaged first while the room is clean, and then the raw products could be packaged. This allows "separation" using process schedules to prevent cross contamination. It is important to note that all of the other facility control steps should be addressed even if scheduling processing times is used as the method for providing "physical separation." A facility that is utilizing a processing schedule to provide the separation must have a strict sanitation program and a process for evaluating equipment prior to use.

2. Sanitation:

All establishments must recognize the importance of the sanitation crew and the activities completed during the sanitation process. The sanitation program must be effectively implemented and appropriate results obtained. It is recommended that producers of RTE products evaluate their Sanitation Standard Operating Procedures (SSOPs) to ensure that they are adequate. Sanitation programs should include:

- Full support of management, including sufficient funding for personnel, equipment, training, and supplies

- Method to establish accountability for the sanitation programs which may include the use of:
    - Microbiological monitoring
    - Coliform plates
    - Standard plate counts
    - Environmental testing for Listeria species
    - Pre-operational ATP testing
    - Visual inspections (organoleptic evaluation)
    - Tracking of chemical usage, types, concentrations and rotation schedules
    - Review of sanitation crew training records

3. Employees:

Establishments should develop procedures for employee practices during the production of RTE products. Issues that establishments should consider include:

- Development of a written procedure for employee hygiene and method for training.
- Employee training on personal hygiene is a crucial component of creating employee behavior that protects the integrity of the RTE products.
- Developing a process for emphasizing the importance of employee hand washing and/or gloving.
- The use of a separate color of frocks designated only for RTE product handling areas, and the use of aprons may be incorporated. Frock colors can also be used to distinguish "product handlers" from "non-product handlers" within the RTE area.
- The use of appropriate footwear (boots) should be required, and procedures for cleaning, storing, evaluating condition, and wearing outside of the RTE area should be established.
- Employee traffic flow must be maintained to prevent cross-contamination. Flow should not allow employees to move from raw to RTE areas without following all of the procedures outlined for RTE personnel.

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Chapter 9

Food Preservation Techniques

Food preservation may be defined as the set of treatment processes that are performed to prolong the life of foods and at the same time retain the features that determine their quality, like color, texture, flavor and especially nutritional value. Food preservation processes have different time scales, ranging from short periods needed for home cooking and cold storage methods, to much longer periods of time required by strictly controlled industrial procedures such as canning, freezing and dehydration.

There are many techniques used to preserve food such as legal food additives, varying levels of food ingredients or components, and new technology. Legal food additives, among other functions, can prevent oxidation and inhibit or destroy harmful microorganisms (molds and bacteria). Vitamin E or vitamin C can serve as an antioxidant in many food products, and benzoate in beverages can act as an anti-microbial agent. We can preserve food by manipulating the levels of food ingredients or components to inhibit the growth of microorganisms or destroy them. For example, keep the food low in moisture content (low water activity), high in sugar or salt content, or at a low pH (less than pH 5).

The preservation of fruits and vegetables entails the partial utilization of the raw material. In some cases, during the process it becomes necessary to add a packing medium, e.g., syrup or brine, while in others the raw material is used alone, as in frozen products. The raw material may be processed differently, depending upon the product to be obtained, e.g. vegetables in sauce, jellies, pickles and juices, for instance. The same raw material may be processed in different ways, as a result of which different products will be manufactured. In general terms, some processing methods are mentioned as below.

- Refrigeration
- Cold storage with modified atmosphere
- Superficial chemical treatments
- Special storage condition
- Packaging systems involving modifications in atmosphere
- Canning
- Freezing
- Dehydration
- Preservation Methods by Chemical Action

Acids, salts and sugars are the principal food preservatives of a chemical nature. Sodium chloride is perhaps the oldest compound serving as a preservative. Acids, mainly lactic, but occasionally including propionic, are produced. Acetic acid in the form of vinegar is used in the manufacture of several pickled products. Benzoic acid, sodium salts - sodium propionate, di acetate and sulfur dioxide, and sodium chloride are added to foods to prevent spoilage. Sugars are employed in the

manufacture of jelly, jams, preserves, sweetened condensed milk, sweet pickles, and other products aiding the preservation of the products into which they are incorporated.

Preservation by the addition of sugar: Sugar is generally added in the processing of jams, jellies and sweet. The fruit must be boiled, after which the sugar is added in variable amounts, depending upon the kind of fruit and the product being prepared. The mixture must then continue to boil until it reaches a high level of soluble solids, which allows for its preservation. The addition of sugar combines with certain fruit substances to produce a gel - like consistency, which characterizes the texture of jams and jellies. To achieve this, appropriate acidity levels and sugar content, together with pectin, form a proper gel.

Benzoic acid and benzoate: Benzoic acid is a stable, white, granular or crystalline powder possessing a sweet, stringent taste. The sodium salt is more soluble in water (62.5 g in 100 ml) at 25° C than benzoic acid. For this reason it is the preferred form for industrial use. Sodium benzoate has an optimum pH range between 2.5 and 4.0. If the pH of the food product is above pH 4.5, acidification may be desirable; the benzoate can be 100 times more active at this optimal pH as compared to pH above 6.0. The microbial level decides the amount of benzoate required. Temperature also plays a part; cold-stored juices need less benzoate. Some fruit juices at 30° C require as much as 0.05% to prevent fermentation. Consequently, juice should be kept cool or have lower numbers of yeast organisms. For preservation of a wide variety of foods, sodium benzoate or benzoic acid is used in amounts of less than 0.1 percent.

Sulfur dioxide: Sulfur dioxide is used to treat fruits and vegetables before and after dehydration to extend the storage life of fresh grapes, and to prevent the growth of undesirable microorganisms during winemaking and the manufacture of juices. Sulfur dioxide is more effective against mold spores and bacteria than against yeast; therefore it is combined with sodium benzoate, which is more effective against yeast, for fruit squashes. Sulfur dioxide is also used as a preservative in manufactured meats, sausage, and soft cheeses. In meat the flesh color is stabilized.

Sulfur dioxide is added to dried fruit in amounts up to 3000 ppm; less in dehydrated vegetables. During their storage, sulfur dioxide slows deteriorate changes, such as severe darkening in color and off-flavors. Its reducing action is valuable in preventing the loss of ascorbic acid in dried fruits and vegetables and the disappearance of beta-carotene (pro-vitamin A) in vegetables. Frequently as much as 90 percent sulfur dioxide is removed by steam during the cooking of dried fruits and vegetables.

Sulfur dioxide is usually applied to vegetables after blanching and before dehydration, in the form of Sodium metabisulphite solution. The uses of sulphites, or sulfur dioxide, to treat vegetables prior to dehydration, aids in the prevention of deteriorative changes during dehydration and storage. Sulfur dioxide is a useful agent for the prevention of browning reactions in dried fruits. It should be declared on the label, for those consumers who may be sensitive to it, e.g. people with an asthmatic condition.

Treatment with acid: Most foods may be preserved by heat treatment when they have a pH lower than 4.0. For this reason several methods have been developed which control the pH through the

production of acid, or the addition of some organic acid, like acetic, citric and even lactic acid. The acidification of low-acidity vegetables to less than pH 4.5 for commercial sterilization-based processing, with brief sterilization periods at temperatures of 100°C, is a very practical method to employ on a small-scale and even home processing.

Raw and processed foods should be packaged to prevent oxidation, microbial contamination, and loss of moisture. Storage of foods (when not contaminated) below -20°C can keep food for several months or a year. Storing foods at 4°C can extend the shelf life to several days or a week (note that some bacteria such as Listeria monocytogenes can still grow and multiply even in foods at refrigerated temperatures).

Today, the demand for processed foods goes beyond the fundamental requirements of safety and shelf-stability. More emphasis is being placed on informatively labeled, high-quality, and value added foods with convenient end use. Improvements in quality and safety of processed foods have been achieved through regulatory requirements on manufacturers, and national or international legislature that recommend and/or enforce performance standards or methods for achieving safety and quality assurance. Equally important is the fact that the need for affordable, yet, high value-added products has been driven by the consumer.

In all its forms of application, thermal processing persists as the most widely used method of preserving and extending the useful shelf life of foods. The concept of in-container sterilization (canning) involves the application of a high-temperature thermal treatment for a sufficiently long time to destroy microorganisms of public health and spoilage concerns. The hermetic seal maintains an environment in the container that prevents the growth of other microorganisms of higher resistance and most importantly, prevents recontamination and pathogens from producing toxins during storage.

Conventional canning operations have the tendency to induce permanent changes to the nutritional and sensory attributes of foods. Therefore, recent developments in food processing operations have aimed at technologies that have the potential to substantially reduce damage to nutrients and sensory components by way of reduced heating times and optimized heating temperatures.

Principles of thermal processing

Thermal destruction of microorganisms is traditionally established to take place following a first order semi-logarithmic rate. Therefore, theoretically, a sterile product cannot be produced with certainty no matter how long is the process time. Targeting a product that is completely void of microorganisms would render the product unwholesome or inferior in quality. Industrially, thermal processes are designed by processing authorities to provide commercially sterile or shelf-stable products. Commercial sterility (as defined by the United States Food and Drug Administration (FDA)) or shelf-stability (U.S. Department of Agriculture (USDA)) refers to conditions achieved in a product by the application of heat to render the product free of microorganisms that are capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution. Designing a sound thermal process requires extensive understanding of process methods, the heating behavior of the product and its impact on a target

microorganism. Thus, the severity of any thermal process must be known and depend on factors such as: (i) the physical characteristics of the food product including thermo-physical properties, shape and size of the container holding the product, (ii) the type and thermal resistance of the target microorganisms that are likely to be present in the food, and (iii) the pH, water activity (aw) and salt content of the food. Due to health-related concerns on the use of salt, there is increased demand to reduce salt levels in foods. The United States Food and Drug Administration (FDA) has classified foods in the federal register as follows: (i) acid foods, (ii) acidified foods and (iii) low acid foods. Acid foods are those that have a natural pH of 4.6 or below. Acidified foods (e.g., beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers, tropical fruits and fish) are low acid foods to which acid(s) or acid foods are added with a water activity greater than 0.85 and a finished equilibrium pH of 4.6 or below. Low-acid foods have been defined as foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity greater than 0.85.

Scientific investigations have revealed that spores of Clostridium botulinum will not germinate and grow in food below pH 4.8. To provide sufficient buffer, a pH of 4.6 has generally been accepted as the point below which C. botulinum will not grow to produce toxin. Thus, a pH of 4.6 represents a demarcating line between low and high acid foods. During thermal processing of low acid foods (pH≥4.6), attention is given to C. botulinum: the highly heat resistant, rod-shaped, spore former that thrives comfortably under anaerobic conditions to produce the botulism toxin. Commercial sterility is achieved when C. botulinum spores are inactivated to satisfy regulatory requirements. However, other heat resistant spores (generally referred to as thermophiles) such as Clostridium thermosaccolyaticum, Bacillus stearothermophilus, and Bacillus thermoacidurans have the potential to cause spoilage and economic losses when processed cans are stored under “abuse” storage conditions of temperature. The thermophiles would be of no consequence provided one can guarantee that processed cans would be stored at temperatures below 30 oC.

Principles of freezing

The freezing process implies two linked processes: (1) lowering of temperature by the removal of heat and (2) a change of phase from liquid to solid. The change of water into ice results in increase in concentration of unfrozen matrix and therefore leads to dehydration and lowering of water activity. Both the lowering of temperature and the lowering of water activity contribute to freezing as an important preservation method.

In order for a product to freeze, the product must be cooled below its freezing point. The freezing point of a food depends on its water content and the type of solutes present. The water component of a food freezes first and leaves the dissolved solids in a more concentrated solution, which requires a lower temperature to freeze. As a result, the freezing point decreases during freezing as concentration increases. Different solutes depress the freezing point to a different degree.

Evaporation and Dehydration

Evaporation: During food processing, evaporation is used to achieve the following goals: (1) concentrate food by the removal of water, (2) remove undesirable food volatiles, and (3) recover desirable food volatiles. Traditionally, evaporation is achieved via the following methods: (1) Use sun energy to evaporate water from seawater to recover the salts left behind.

(2) Use a heated kettle or similar equipment to boil water from liquid or semisolid foods (e.g., sugar syrup). (3) An improved method is to evaporate under a vacuum. The term "vacuum evaporator" refers to a closed heated kettle or similar equipment connected to a vacuum pump. One principle to remember is that a major objective of vacuum evaporators is to remove water at temperatures low enough to avoid heat damage to the food.

Drying

Drying differs from evaporating in that the former takes the food to nearly total dryness or the equivalence of 97 or 98% solids. The oldest method of drying food is to put the food under a hot sun. This practice probably started thousands of years ago. Although sun drying is still practiced, especially in many third world countries, modern food drying has been modified to a nearly exact science. Drying has multiple objectives: (1) to preserve the food from spoilage, (2) to reduce the weight and bulk of the food, (3) to make the food enjoy an availability and consumption pleasure similar to that of canned goods, and (4) to develop "new" or "novelty" items such as snacks. Some well-known products prepared from drying include dried milk powder, instant coffee, fish and shell fish, jerky, dried fruits, and dried potato flakes.

Food Additives

One popular method of food preservation uses chemicals, legally known as food additives. The main functional objectives of the use of food additives are (1) to keep bread mold free and salad dressings from separating, (2) to help cake batters rise reliably during baking and keep cured meats safe to eat, (3) to improve the nutritional value of biscuits and pasta and give gingerbread its distinctive flavor, (4) to give margarine its pleasing yellow color and prevent salt from becoming lumpy in its shaker, and (5) to allow many foods to be available year round, in great quantity and the best quality. Food additives play a vital role in today's bountiful and nutritious food supply. They allow our growing urban population to enjoy a variety of safe, wholesome, tasty foods year-round. And they make possible an array of convenience foods without the inconvenience of daily shopping. Although salt, baking soda, vanilla, and yeast are commonly used in foods today many people tend to think of any food additive as a complex chemical compound. All food additives are carefully regulated by federal authorities and various international organizations to ensure that foods are safe to eat and are accurately labeled. The purpose of this section is to provide helpful background information about food additives, why they are used in foods and how regulations govern their safe use in the food supply.

Additives are used in foods for five main reasons:

1. To maintain product consistency. Emulsifiers give products a consistent texture and prevent them from separating. Stabilizers and thickeners give smooth uniform texture. Anticaking agents help substances such as salt to flow freely.

2. To improve or maintain nutritional value. Vitamins and minerals are added to many common foods such as milk, flour, cereal, and margarine to make up for those likely to be lacking in a person's diet or lost in processing. Such fortification and enrichment have helped reduce malnutrition in the U.S. population. All products containing added nutrients must be appropriately labeled.

3. To maintain palatability and wholesomeness. Preservatives retard product spoilage caused by mold, air, bacteria, fungi, or yeast. Bacterial contamination can cause food-borne illness, including life-threatening botulism. Antioxidants are preservatives that prevent fats and oils in baked goods and other foods from becoming rancid or developing an off flavor. They also prevent cut fresh fruits such as apples from turning brown when exposed to air.

4. To provide leavening or control acidity/ alkalinity. Leavening agents that release acids when heated can react with baking soda to help cakes, biscuits, and other baked goods to rise during baking. Other additives help modify the acidity and alkalinity of foods for proper flavor, taste, and color.

5. To enhance flavor or impart desired color. Many spices and natural and synthetic flavors enhance the taste of foods. Colors likewise enhance the appearance of certain foods to meet consumer expectations.

Table 2. Common additives used in various food items

Common Uses of Additives **Additive Functions/Examples**	**Foods Where Likely Used**
Impart/maintain desired consistency • Alginates, lecithin, mono and di glycerides, methyl cellulose, pectin, guar gum,	Baked goods, cake mixes, salad dressings, ice creams, cheese, coconut, table salt
Improve/maintain nutritive value • Vitamin A and D, thiamine, niacin, riboflavin, folic acid, ascorbic acid, calcium carbonate, iron, zinc oxide	Flour, bread, biscuits, breakfast cereals, pasta, milk, margarine, iodized salt, gelatin desserts
Maintain palatability and wholesomeness • Propionic acid and its salts, ascorbic acid, butylated hydroxyl anisole (BHA), benzoates, sodium nitrite, citric acid	Bread, cheese, crackers, frozen and fried fruits, potato chips, cake mixes
Produce light texture; control acidity/alkalinity • Yeast, sodium bicarbonate, citric acid, fumaric acid, phosphoric acid, lactic acid, tartrates,	Cakes, cookies, quick breads, crackers, butter, chocolates, soft drinks
Enhance flavor or impart desired color • Cloves, ginger, fructose, aspartame, saccharin, monosodium glutamate, caramel, annatto, turmeric, limonene	Spice cake, ginger bread, soft drinks, yogurt, baked goods, soup, cheeses,

Packaging and Packaging Materials

If the climate is very dry, it may not be necessary to package dried foods as they may not pick up much moisture from the air. However a humid climate results in dried foods gaining moisture. The stability of dried foods depends not only on the humidity of the air, at which a food neither gains nor loses weight, but also on the type of food. Different foods can be grouped according to their ability to absorb moisture from the air. The two groups are hygroscopic, which absorb moisture easily and non-hygroscopic, which do not absorb moisture. This difference determines the packaging requirement for different fruit and vegetable products. The moisture content at which a dried food is stable is known as the Equilibrium Moisture Content and examples of this for different fruits and vegetables are available, together with the packaging requirement for different groups of foods. Dried fruits and vegetables are usually packaged in one of the many different types of plastic film. The selection of the correct type of packaging material depends on a complex mix of considerations.

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UNITS, MEASUREMENTS, CONVERSIONS AND USEFUL DATA

SI prefixes and multiplication factors

Multiplication factor		Prefix	Symbol
1 000 000 000 000 000 000 000 000	$=10^{24}$	yotta	Y
1 000 000 000 000 000 000 000	$=10^{21}$	zetta	Z
1 000 000 000 000 000 000	$=10^{18}$	exa	E
1 000 000 000 000 000	$=10^{15}$	peta	P
1 000 000 000 000	$=10^{12}$	tera	T
1 000 000 000	$=10^{9}$	giga	G
1 000 000	$=10^{6}$	mega	M
1 000	$=10^{3}$	kilo	k
100	$=10^{2}$	hecto	h
10	$=10^{1}$	deca	da
0.1	$=10^{-1}$	deci	d
0.01	$=10^{-2}$	centi	c
0.001	$=10^{-3}$	milli	m
0.000 001	$=10^{-6}$	micro	μ
0.000 000 001	$=10^{-9}$	nano	n
0.000 000 000 001	$=10^{-12}$	pico	p
0.000 000 000 000 001	$=10^{-15}$	femto	f
0.000 000 000 000 000 001	$=10^{-18}$	atto	a
0.000 000 000 000 000 000 001	$=10^{-21}$	zepto	z
0.000 000 000 000 000 000 000 001	$=10^{-24}$	yocto	y

SI base, derived and other units

Phisical quantity	Unit	Symbol
length	metre	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd
time	minute	min
time	hour	h
time	day	d
plane angle	degree	º
plane angle	minute	’
plane angle	second	’’
length	foot	’
length	inch	’’
length	angstrom	A
area	barn	b
volume	litre	l
mass	tonne	t
pressure	bar	bar

SI Units

Length and area

Micron (μm, μ)	$=10^{-6}$ m
Angstrom (A)	$=10^{-10}$ m
Fermi (fm)	$=10^{-15}$ m
Are (a)	$=100$ m^2
Barn (b)	$=10^{-28}$ m^2

Mass

Tonne (t)	$=10^6$g$=1000$kg

fps Units

Length

12 inches	=1 foot (ft)
3 feet	=1 yard (yd)
22 yards	=1 chain
10 chains	=1 furlong
8 furlongs	=1 mile (mi)
6 feet	=1 fanthom
6080 feet	=1UK nautical mile

Area

4840 yard^2	=1 acre
640 acres	=1 mile^2

Mass

16 ounces (oz)	=1 pound (lb)
14 pounds	=1 stone
28 pounds	=1 quarter
4 quarters	=1 hundredweight
20 hundredweight	=1 ton

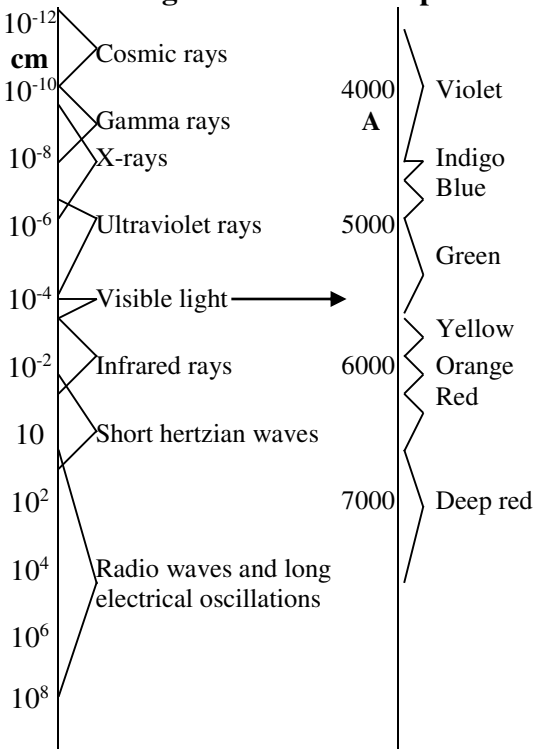
Volume

20 fluid ounces	=1 pint (pt)
2 pints	=1 quart (qt)
4 quarts	=1 gallon

Conversion Table

fps Units	SI Units	Reciprocal
Length		
1 inch (in)	$=2.54 \times 10^{-2}$m	39.370079
1 foot (ft)	=0.3048 m	3.280839
1 yard (yd)	=0.9144 m	1.093613
1 fanthom	=1.8288 m	0.546806
1 chain	=20.1168 m	4.97097×10^{-2}
1 furlong	$=2.01168 \times 10^2$ m	4.97097×10^{-3}
1 mile (mi)	1.609344×10^3 m	6.213712×10^{-4}
Area		
1 in^2	$=6.4516 \times 10^{-4}$m^2	1.550003×10^3
1 ft^2	$=9.290304 \times 10^{-2}$m^2	10.763910
1 yd^2	=0.836127 m^2	1.195990
1 mi^2	$=2.589988 \times 10^6$ m^2	3.861022×10^{-7}
1 acre	$=4.046856 \times 10^3$ m^2	2.471054×10^{-4}
Volume		
1 in^3	$=1.638706 \times 10^{-5}$ m^3	6.102374×10^4
1 ft^3	$=2.831685 \times 10^{-2}$m^3	35.31467
1yd^3	=0.764555 m^3	1.307950
1 fluid ounce (fl oz)	$=2.841306 \times 10^{-5}$m^3	3.519508×10^4
1 pint (pt)	5.682613×10^{-4}m^3	1.759754×10^3
1 quart (qt)	$=1.136523 \times 10^{-3}$m^3	8.798770×10^2
1 gallon (gal)	$=4.54609 \times 10^{-3}$m^3	2.199692×10^2
1 bushel (bu)	=0.036369 m^3	27.495944
1 US gallon (=231in^3)	$=3.785412 \times 10^{-3}$ m^3	2.641721×10^2
Mass		
1 ounce (oz)	$=2.834952 \times 10^{-2}$ kg	35.273962
1 pound (lb)	=0.45359237 kg	2.204623
1 stone	=6.350293 kg	0.158473
1 quarter	=12.700586 kg	7.873652×10^{-2}
1 hundredweight	=50.802345 kg	1.968413×10^{-2}
1 ton	$=1.016047 \times 10^3$ kg	9.842065×10^{-4}

Electromagnetic and visible spectra



Designation of large numbers

	USA	UK
10^6	million	million
10^9	billion	milliard
10^{12}	trillion	billion
10^{15}	quadrillion	billiard
10^{18}	quintillion	trillion

Conversion Factors

Parameter	Conversion	Reciprocal
P_2O_5	=P x 2.29	0.43668
K_2O	=K x 1.2	0.83333
Urea	=N x 2.174	0.46
SSP	=P_2O_5 x 6.25	0.16
MOP	=K_2O x 1.67	0.275438
Organic matter %	=C x 1.74	0.5747

Soil test rating

Parameter	Low	Medium	High
O.C. %	< 0.5	0.5-0.75	> 0.75
N kg/ha	< 280	280-560	> 560
P kg/ha	< 10	10-25	> 25
K kg/ha	< 110	110-280	> 280

Useful data

1 atmo = 764 mm Hg = 1036 cm water = 1013 mbar = pF 3 = 1.03329 kg/cm^2 = 1013231 dynes/cm^2
1 ha = 0.01 km^2 = 10000 m^2 = 2.471 ac = 0.003861 mile^2
1 ac = 100 cents = 0.4 ha = 4047m^2 = 43560ft^2 , 1 cent = 40.47m^2 = 436ft^2
1 mile = 8 furlong = 80chains = 1760yards = 5280ft
1 ton = 20 hundredweight (cwt) = 80 quarter = 2240 pounds = 35840 ounces
1 HP = 76.0404 kg.m/s = 745.7 watts = 550 ft.lb/s
Erosion: v velocity, v^2 erosive power, v^5 amount eroded, v^6 size of materials carried away.
When slope is increased 4 times: increase in velocity 2 times, erosivity 4 times, quantity 32 times, size 64 times.
Construct bunds every 3 ft vertical drop or 300 ft length whichever is less
Solar constant: 2cal/cm^2/minute. Average energy received on earth from sun.
Plants use only 0.4-0.5% energy. Algae use the maximum, 2.5%
Beer's law $I=I_oe^{-KL}$
Hopkins bioclimatic law: Crop phenological events are delayed by 1 day for every 1° latitude, 5° longitude and 400 ft altitude.
15 cm 1 ha furrow slice = 2.2 million kg
1 ha 1 mm water = 10 m^3
1 cusec (cubic foot/s) = ft^3/s = 28.3 l/s = 1 acre inch/hour
1 cumes (cubic metre/s) = m^3/s = 35.3 cusecs
°C = (°F – 32) 5 ÷ 9 ; °F = 1.8°C + 32
Balanced fertilizer application based on balanced ratio of NPK 4:16:1 in the economic part.
Protein contains 16% N, Protein % =N% x 6.25
Organic matter contains 58% carbon, Organic matter % =C % x 1.724
Soil water potential, $\psi = \psi_{m/p} + \psi_{o/s} + \psi_g + \psi_a$
Pan evaporation, E_o=4-6 mm/day, $Et_o=E_o$ x 0.6-0.8
Irrigation requirement: depth=3-8cm, interval=8-10days, IW/CPE=0.9 for sensitive crops, 0.6 for hardy crops
N x Eq. wt=g/l, N=Eq. wt/l, ppm=µg/ml=mg/l=me/l x Eq. wt.
mmhos/cm, EC x 640=TSS, ppm; EC x 0.064=TSS%; EC x 10=TSS, me/l; EC x –0.36=$\psi_{o/s}$ (+ osmotic pressure)
NPK recovery by crop: <40, <20, 80-90%
Cation adsorption to clay: Al>Fe>Si>H>Ca>Mg>K>Na
Anion adsorption to clay: SiO_4>PO_4>MO_4>SO_4>NO_3>Cl
(Cation and anion leaching in reverse order)
Anion toxicity to crops: HCO_3>CO_3>Cl>SO_4>NO_3
Anaerobic reduction during flooding: O>NO_3>Mn>Fe>S>C
Lyophilic series/Displacement capacity of anions: F>OH>HCO_3>PO_4>SiO_4
Plant mobile nutrients: N, P, K, Mg, Cl, S
Plant immobile elements: Ca, Fe, Mn, Zn, Cu, Mo, B
Particle size: solution < 10A colloid 1000A > suspension
Neutralising value (Ca equivalent): $CaCO_3$ 100, $MgCO_3$ 119, $Ca(OH)_2$ 136, CaO 179
Residual (equivalent) acidity: $CaNH_4NO_3$ zero, Urea 80, NH_4NO_3 60, $(NH_4)_2SO_4$ 110, $(NH_4)_3PO_4$ 86, NH_4Cl 128, anhydrous NH_3 148
Essential elements: C, H, O, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B, Cl, Co
Soils having >20% o.m. = organic; >70% sand = sandy; >40% clay = clayey; 27-52% silt = silty soil