CHEMICAL QUALITY ASSURANCE

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Chemical Quality Assurance

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Champions aren't supernatural,
They just fight 1 more second
When everyone else quits.
Sometimes 1 more second of effort gives you the victory of life time….

EDITOR

Course Outlines 3(2+0+2)

Module 1: INTRODUCTION TO CHEMICAL QUALITY ASSURANCE
Lesson 1. Concept and importance of chemical quality control in dairy industry
Lesson 2. Hazard analysis and critical control points (HACCP)

Module 2: LEGISLATION AND STANDARDS OF MILK AND MILK PRODUCTS
Lesson 3. National regulations: PFA, Agmark, BIS and FSSAI
Lesson 4. International regulations: Codex, IDF, FDA, ISO, EEC

Module 3: CHEMICAL ANALYSES OF MILK AND MILK PRODUCTS
Lesson 5. Terminology of standard solutions and reagents
Lesson 6. Preparation and standardization of solutions
Lesson 7. Sampling procedures; labeling of samples for analysis and choice of analytical tests
Lesson 8. Detection of neutralizers, preservatives and adulterants in milk and milk products
Lesson 9. Analysis of market samples of milk and milk products
Lesson 10. Instrumental methods of analysis
Lesson 11. Prediction of shelf life behavior of milk and milk products

Module 4: ENVIRONMENTAL CONTAMINANTS IN MILK AND MILK PRODUCTS
Lesson 12. Pesticides and Antibiotics
Lesson 13. Heavy metals
Lesson 14. Methods of estimation of Pesticides, Antibiotics and Heavy metals
Lesson 15. Milk contact surfaces

Module 5: WATER, DETERGENTS AND SANITIZERS FOR DAIRY PLANT OPERATIONS
Lesson 16. Soft and hard water, temporary and permanent hardness
Lesson 17. Softening of hard water
Lesson 18. Detergents and sanitizers

Module 6: SETTING-UP OF LABORATORY FOR CHEMICAL ANALYSIS OF MILK AND MILK PRODUCTS
Lesson 19. Setting up quality control laboratories, testing facilities and Mobile testing laboratories
Lesson 20. Calibration of laboratory glassware: butyrometer, pipettes, burettes, lactometers and thermometer
Lesson 21. Accreditation of analytical laboratories

Lesson 1

CONCEPT AND IMPORTANCE OF CHEMICAL QUALITY CONTROL IN DAIRY INDUSTRY

1.1 Introduction

The Indian Dairy Industry has acquired substantial growth in the recent years. Milk group contributes highest to the total output of the agricultural sector. Despite all these facts, we are not able to make a dent in the international market. Our export is still far- far below than the countries, whose milk production is less. The obvious reason for this is the quality of our raw milk and thereby the quality of the finished products. Most of the dairy plants are still sticking to the old system maintaining their quality, without considering the developments made in the tools to attain a consistent quality of any food product. Rising liberalization of agro-industrial markets and the world-wide integration of food supply chains require new approaches and systems for assuring food safety. At present, concern over food safety is at an all-time high. In response, the public and the private sector have developed new process standards and require suppliers of food products to follow them. Both, the market and legislations in importing countries demand for comprehensive and transparent schemes reaching from "farm to fork" and "boat to throat" Organizations in the food sector will need to manage risk, demonstrate good corporate responsibility and meet legal requirements if they are to remain competitive, protect their reputation and enhance their brand. Hence, quality management tools like *Hazard analysis and critical control points (HACCP), ISO 22000:2005 - Food Safety Management Systems,* Six Sigma etc are very much relevant in the present era of Globalization and aware consumers.

1.2 Importance of Chemical Quality Control In Dairy Industry

Milk is a highly perishable commodity. This perishable character of milk makes the life tough for the milk handlers, especially in the absence of proper infrastructure of cooling and erratic power supply. Since, dairy animals are fed on agricultural by products and crop residues, therefore the contaminant present in the animal get secreted along with milk in the form of pesticide and veterinary drug residues. These contaminants not only affect the health of the consumers, but also the quality of the products especially fermented products. Moreover, some of the unscrupulous persons involved in this trade are further tarnishing the image of Indian dairy industry by adulterating milk with various chemicals, which are injurious to health. In the wake of all these facts, quality control of milk assumes very high importance and priority. The chemical quality control in dairy industry will enable the industry in the following ways:

- The strict quality control both chemical and microbiological will deter the adulteration of milk and will improve the quality of raw milk.
- The improvement of the raw milk quality through regular checks can improve the quality of the milk products prepared from such milk.
- It will install the confidence in national consumers, who are drifting away from the indigenous milk products.
- It will also increase the export potential of Indian milk products, and finally the National image with reference to Indian milk quality.
- The strict quality control will also increase the revenue of a dairy producer.

1.3 Quality Control and Quality Assurance

As per ISO: 8402 (1994): Quality is the totality of features and characteristics of a product or service that bear (determine) on its ability to satisfy stated or implied needs (requirements)"

$$Quality \propto 1/variability$$

"*Quality control* (QC) involves the set of activities used to ensure that the products and services meet / fulfill requirements for quality"

Traditionally Q.C is a laboratory function only and is related to analysis of samples i.e testing and judging of raw materials/ and finished products for acceptance or rejection.

1.3.1 Purpose & Aim

To ensure that products are within the well defined and accepted standards thereby protecting the legal and health rights of consumers and financial interests of producers / manufacturers.

1.3.2 Limitations

Recall of products is more because products are tested in the last stage or as finished products. This ultimately wears the impact on reputation of the company.

"*Quality assurance (QA)* is the set of activities which ensures that the quality levels of products and services are properly maintained and that the supplier and customer quality issues are properly resolved"

Quality assurance gives adequate confidence that product or service will satisfy given requirements for quality.

1.3.3 Purpose/Aim

1.3.3.1 Internal purpose

Within an organization QA provides confidence to the management.

1.3.3.2 External purpose

Outside the organization provides confidence to consumers or others. Compared to QC, QA is much wider in the sense, it demands full control over the quality of raw materials, control over the process at different levels and control over distribution set up etc.

Notion is that "Prevention rather than Detection". It is a proactive approach rather than a reactive approach.

OBJECTIVES AND IMPORTANCE OF QUALITY ASSURANCE

—To maintain legal standards and legal requirements
—To fulfill customer's requirement in terms of various attributes

- Physical (body, texture, colour, etc)
- Chemical composition

- Microbiological
- Safety
- Consumers should get what they pay for
- This leads to increased consumer satisfaction and less complaints

—To check adulteration in incoming material in order to prevent substandard product, hazards or problems in the process
—To check efficiency of processes: heating, cooling, removing hardness from water, effluent treatment etc
—To safeguard nutritive value of milk and milk products
—To check wastage of material
—To help in research and developments
—To ensure general cleanliness and sanitation in factory premises

BENEFITS

- Reduction in unit cost of production
- Reduction in wastage and scrape
- Less complaints from customer
- Avoids repeated inspection
- Increases production since rejection reduces
- Efficiency of unit goes up
- Management gets proud place in society
- Boost employee's morale
- Reduction in production bottlenecks

ROLE OF QUALITY ASSURANCE DEPARTMENT

- Sanitation- defines requirements for cleaning and sanitary activity and their monitoring
- Sanitation standard operating procedure (SSOP)
- Standard operating procedure (SOP)
- Good manufacturing practices (GMP)
- Foreign material control
- Quality control- Chemical and Microbiological testing
- Documentation control
- Pest control
- Hazardous material control
- Allergen Protocol for controlling allergenic material
- Record control- identification and maintenance
- Calibration
- Water quality and water treatment programmes
- Sensory training and sensory evaluation
- Supplier certification and ongoing supplier evaluations
- Receiving, storage and control of raw ingredients and packaging material
- Control of non-conforming product and process
- Product identification traceability and Product recall
- Handling customer's complaints
- Labeling- application and control of labels
- Preventive maintenance
- Formalized management review process
- Waste water (effluent treatment programme)

- Training
- Corrective/preventive action- root cause analysis and follow up evaluation to confirm effectiveness of action taken
- Internal auditing

Why focus on quality?

1. Globalization/ liberalization

Globalization has changed the way businesses are done. Availability of the number and variety of products has increased in the market. After the liberalization (after 1991) in India several players including multinationals have entered into food business. This has increased the level of competition. If one has to sustain the competition he can't do so with the substandard products.

2. Technological advances in food processing (e.g. Nanotechnology, Genetic engineering)

The applications of modern science and biotechnology for food production and genetically modified foods and crops have presented novel opportunities and made enormous contributions in agriculture and food production. Certain technological development has also posed risks to food safety due to insufficient knowledge. Like Genetically modified organisms (GMO)/ Genetically modified foods and use of Nano technology for food production. These technologies are controversial and a cause of concern since we still have limited knowledge of their impact on human health for current and future generations and on natural ecosystems.

Advancement of technology has also affected food safety in positive manner. Development latest instruments like biosensors, Nano Sensors in Pathogen detection and various chromatographic techniques which helps in identifying food safety hazards.

Several advances made in the areas like maintaining nutritional quality, increasing the shelf life of foods, better transportation and storage facilities, better packaging materials etc. has potential to enhance the safety and quality of food.

Emergence of functional food is also an area which can affect the food safety. Due to their diversity all functional foods require a case by case evaluation for their safety.

3. New hazards and concerns

Furthermore, higher levels of environmental pollution in our soil, water, and air are increasing the presence of contaminants such as toxic metals, dioxins, and polychlorinated biphenyls (PCBs) in food. Also, several substances designed to increase overall food production, such as insecticides, veterinary therapeutics, and hormones, are affecting the quality and content of food being produced, often with poorly understood long-term consequences.

In addition certain hazards like Bisphenol A in certain packaging materials (e.g. polycarbonate), *E.coli* 0157:H7, trans fat in formulated foods, acrylamide in fried products, fungal toxins like aflatoxin can affect food safety adversely and hence endanger quality of food.

4. Increased consumer awareness

Due to increased level of literacy and better communication facilities consumer has become aware and conscious about the quality and safety of food he consumes. Healthy, nutritious, safe and better quality products are therefore the need of the hour.

5. Stricter regulatory frame works

Regulatory bodies of the worlds including India are becoming stricter and science based. This has led to adoption of better processing technologies and measures which ensures good quality food to the consumers by food processor. New laws are passed considering new threats to the food safety and changed nature of food business worldwide.

Evolution of Quality Concept

Historically control of quality in food parallels the history of food production. From the earliest beginnings every food manufacturer, handler and consumer attempted to evaluate and control the quality of the food he used. Initially various attributes of quality were measured and decisions made on the basis of sensory evaluation. With development of chemical and instrumental methods for evaluating food quality and statistical methods for interpretation of results, work in the area of quality control expanded. Now quality control has grown as a distinct discipline. All concepts of quality, emerged over a period of time are presented below in a chronological order.

Craftman (<1900)

- Inspection of a product was not distinguished from the task of producing it.
- Each craftman was inspecting his own work to avoid customer's complain.

Operator (1900 – 1910)

- Due to industrial revolution machines were employed for the production and craftman became operator of the machine.
- Since the machines were not able to inspect its own output, the operator became the inspector of the quality.

Foreman (1910 – 1920)

- When there were several machines & operator foreman (supervisor) was appointed for supervision of the production work.
- It became the responsibility of a foreman to inspect and decide the quality.

Inspection (1920 – 1940)

- During this era, responsibility for judging the quality was given to inspector.
- There was little or no planning for determining whether the product was right or not.
- Term often used hundred percent (100%) which mean differently to different people.

Statistical Quality Control (SQC)

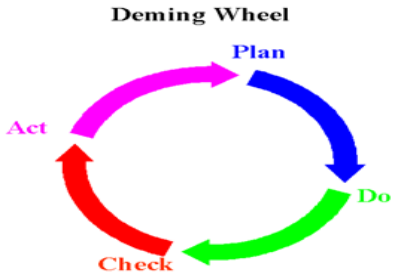
- During the World War II, there was a tremendous requirement for mass production of weapons and other materials, which necessitated the concept of Quality control by making use of statistical tools. This use of statistical tools in controlling the processes was termed as Statistical Quality Control.
- This was an extension of the inspection phase where inspectors provided with a few statistical tools, such as sampling plans & control charts.
- The most significant contribution was sampling inspection, rather than total inspection.
- It is the collection, analysis and interpretation of data to solve a particular problem.
- SQC concept provides a basis for determining a good or acceptable process behavior model

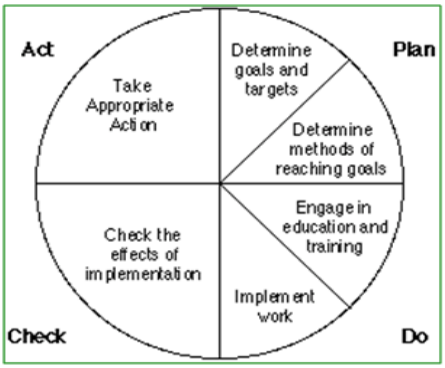
- Any deviation from the model can be traced, identified & eliminated from a process, so that it continues to produce product of acceptable quality.

Deming's Philosophy: Adward Deming proposed his concept of quality assurance. According to which, it involves both process monitoring and eliminating the causes of unsatisfactory performance at all stages.

- It is the name given to entire cycle of activities through which the fitness for use of process, product or service is achieved, with a view to carry out a company's quality function in accordance with the laid down quality objective and policies.
- has divided quality control into four activities i.e.

1) Plan 2) Do 3) Check and 4) Act. Which is known as Deming's PDCA cycle/wheel.





Plan

- Establish goals
- Standardize working procedures
- Train employees

Do

- Carry out the work according to plan

Check

- Verify for compliance with the plan

Act

- In case of non-compliance, find out and remove its root cause(s)

- The Deming's PDCA cycle applies to all situations and area where "quality control' is needed (wanted).
- It is the universal model and covers all activities relating to "Quality Control" , "Quality Assurance" as well as "Quality Improvement".

Quality Control (QC)

- During the passage of time a separate 'Quality control department' came into existence in each factory
- The QC department devoted itself primarily to quality functions.
- Role of QC department was to examine the finished product with a view to verify whether it fulfilled requirements for quality or not.

Total Quality Control (TQC)

- Quality functions cannot be truly achieved by QC department alone
- The department depends on the help rendered by almost all other departments of the company to achieve the goals

—Field force determines the quality needs of the consumers (users)
— The research and development department creates a product concept which can meet these needs
—Chemist or design engineer than prepare product and material specifications suitable for the required quality and specify procedures and instruments to measure the quality
—Purchase department procured material of the right quality
—Plant operators use the processes and equipments to manufacture product
—Inspectors measure the quality attributes and determine fitness of the product for use.
—Sales department market the product and take care at pre-sale stage, on-sale stage and after sale stage.
—Field force gives consumers' reaction, i.e. it creates opportunities for the product improvement which in turn restarts the whole cycle of activities.

- TQC is an integrated organizational approach to delight customers by meeting their expectations on a continuous basis through involvement of everyone in the organization.
- It is an effective system of integrating quality development, quality maintenance and quality improvement efforts of various groups in an organization so as to provide product or service at the most economical levels and which meet full customers' satisfaction.
- It helps in minimizing rejection and rework.

Quality Assurance (QA)

- ISO:8402 (1994)- it comprises the planned and systematic activities implemented within the quality system to provide adequate confidence that a given entity (process/product/service) will fulfill requirements for quality
- All QA activities serve to build confidence internally among the management of organization and externally among its customers and authorities.
- To build confidence quality assurance has to be built into the process; which includes creating records, documenting plans, documenting specifications and reporting reviews.

Such activities and documentation serves to control quality as well as assure it.

Total Quality Management (TQM)

- TQM may be defined as an integrated organizational approach in delighting customers by meeting their expectations on a continuous basis through everyone involved with the organization working on continuous improvement in all spheres namely-process, products and service along with proper problem solving methodology.
- Tools = SQC, QC, TQC, QA : often solve problem in one area of business such as quality of supply or excellence in manufacturing
  So what is required is ?

—a process designed to focus on customer expectation
— preventing problems
—building commitment to quality in workforce
—promoting open decision making

- TQM is a journey- it is the path as well as the goal.

Basic principles of TQM

—Be customer focused – place the customer at the centre of everything you do
—Do it right first time and every time- quality first and always
— Continuous improvement – by using the tool of PDCA in every aspect of work
—Communicate and educate

- Improve communication means tell the people what is going on
- Educate- train the people and retain them

—Measure and recorded

- While finalizing the goals, the Quality indicators (measurements) should be finalized.
- Record the measures as per prescribed documentation
- It allows the company to make decisions based on facts, not opinion.

—Do it together- introduce team working

- Reduces conflict and in-fighting and increases trust and respect
- Bitting problems with wider range of skill – therefore better and more balance solution

Elements of TQM and Integrated TQM Model

The TQM model has three major area

Total Employee Involvement (TEI)

- Include- transformation, Kiazen (small improvement), Hoshin Kanri (Policy development), small group activity, etc

Total Waste Elimination (TWE)

- Include the concept of segregation, arrangement, cleanliness, maintenance or standard and discipline for everything (men and material) i.e. house Keeping.
- Follow the principle of JIT (Just-In-Time), so minimum inventories are maintained.

Total Quality Control (TQC)

- Include – SQC, PDCA cycle, HACCP, QSM (ISO-9000)

Benefits of TQM

- TQM can bring several benefits for consumers, company and employee, if implemented properly.

For customers

- Greater care
- Value for money
- Greater satisfaction
- Better availability
- Result in better customer loyalty.

For company (organization)

- Continuous improvement in quality
- Reduction in cost
- Increase in productivity
- Better motivated work force
- Defects are reduces
- Faster solution of problems

Result in increased cash flow and net profit

for employees

- Empowerment
- More respect
- More training and better skill
- Appreciation and recognition
- **Work satisfac**

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Lesson 2
HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP)

2.1 Introduction

A system was needed that enabled the production of safe, nutritional products for use by National Aeronautics and Space Administration (NASA) starting in the late 1950's to feed future astronauts. So, the Pillsbury company, in 1959, embarked on work with NASA to develop a process of identifying the critical points in the process at which these hazards were most likely introduced into product and therefore should be controlled. The acronym HACCP, which stands for Hazard Analysis and Critical Control Point, is one which evokes 'food safety'. Originally it was developed to ensure microbiological safety of foodstuffs, but now HACCP has been broadened to include chemical and physical hazards in foods. The recent growing worldwide concern about food safety by public health authorities, consumers and other concerned parties, and the continuous reports of foodborne outbreaks have been a major impetus in the application of the HACCP system. HACCP is a systematic preventative approach to food safety that addresses physical, chemical and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCP's) can be taken to reduce or eliminate the risk of the hazards being realised. The system is used at all stages of food production and preparation processes.

2.2 Necessity of HACCP

Food safety has been of concern to humankind since the dawn of history, and many of the problems encountered in our food supply go back to the earliest recorded years. Many rules and recommendations advocated in religious or historical texts are evidence of the concern to protect people against foodborne diseases and food adulteration. However, in recent decades this concern has grown. There are many reasons for this as follows:

• Foodborne diseases remain one of the most widespread public health problems in the contemporary world, and an important cause of reduced economic productivity, despite progress in food science and technologies. The World *Declaration on Nutrition*, adopted by the FAO/WHO International Conference on Nutrition (Rome, December 1992), emphasizes that hundreds of millions of people suffer from communicable and noncommunicable diseases caused by contaminated food and water.

• The increasing incidence of many foodborne diseases, e.g. salmonellosis and campylobacteriosis, in many regions of the world.

• Increased knowledge and awareness of the serious and chronic health effects of foodborne pathogens.

• The possibility of detecting minute amounts of contaminants in food, due to advances in scientific and analytical methods.

• Emerging foodborne pathogens, e.g. *Listeria monocytogenes*, verocytotoxin producing *E. coli*, *Campylobacter spp*, foodborne nematodes, etc.

• An increase in the number of vulnerable people, such as the elderly, immune compromised

individuals, the undernourished, and individuals with other underlying health problems.

• Increased awareness of the economic consequences of foodborne diseases.

• Industrialization and increased mass production, leading to increased risks of food contamination.

• The considerably larger numbers of people affected in foodborne disease outbreaks as a result.

• Urbanization, leading to a more complex food chain, and thus greater possibilities for food contamination.

• New food technologies and processing methods, causing concern either about the safety of the products themselves or the eventual consequences due to inappropriate handling during preparation in households or food service/catering establishments.

• Changing lifestyles, depicted by an increasing number of people eating outside the home, in food service or catering establishments, at street food stalls, or in fast-food restaurants.

• Responsibility for food preparation shared between family members who are not always aware of food safety rules.

• Increased worldwide tourism and international trade in foodstuffs, leading to a greater exposure to food borne hazards from other areas.

• Increased contamination of the environment.

• Increased consumer awareness of food safety.

• Lack of or decreasing resources for food safety.

In the light of the above reasons, there is a increasing concern about food safety, the lack of sufficient resources, and the recognition of the limitations of traditional approaches to food safety assurance which have accentuated the need for a cost-effective food safety assurance method. The HACCP system has proven to be such a system.

2.3 HACCP Terminology

a) Hazard

A biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

b) Contamination

Exposure of food products to hazards, which can cause illness, disease, or even death.

c) Control (verb)

To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

d) Control (noun)

The state wherein correct procedures are being followed and criteria are being met.

e) Control measure

Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

f) Corrective action

Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

g) Control Point

Any step at which biological, chemical, or physical factors can be controlled.

h) Critical Control Point (CCP)

An Essential Point at which Control can be applied so that a Food Safety Hazard can be PREVENTED, ELIMINATED, or REDUCED to an Acceptable Level. It is the last step in the flow of food where a hazard can be controlled.

i) Critical Limit

A maximum and/or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard.

j) Deviation

Failure to meet a critical limit.

k) Flow diagram

A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

l) HACCP (Hazard Analysis Critical Control Point)

A system designed to identify, evaluate, and control of the potential food safety hazards.

m) HACCP Plan

The written document to describe the procedures based on the principles of HACCP and specific conditions.

n) Risk

Probability that conditions will lead to a hazard.

o) Prerequisite Programs

Procedures, including Good Manufacturing Practices that address operational conditions providing the foundation for the HACCP system.

p) Monitor

To conduct a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification.

q) Corrective Action

Procedures followed when a deviation occurs.

r) Step

A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

s) Validation

Obtaining evidence that the elements of the HACCP plan are effective.

t) Verification

Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan.

2.4 What is Hazard?

It is the potential to cause harm to the consumer (the safety aspect) or the product (spoilage aspect).The hazard associated with food safety can be of physical (extraneous matter), chemical (pesticides, insecticides, radionucleides, carcinogenic components, allergens) and biological nature (pathogens, microbial toxins).

2.4.1 Classification of hazards

Based on process and basic ingredients hazards can be classified as follows:

- A food product containing sensitive ingredient
- Manufacturing process does not contain controlled processing step
- There is substantial potential for abuse in distribution or in consumer handling

On the basis indicated above with different combinations of hazard class, as positive (+) or no hazard as (0) is designated and then it is categorized as

Category 1

Special category for products mean for sensitive consumers like baby foods.

Category 2

Which contain either of two hazards as given above

Category 3

When one hazard is present

Category 4

When no hazard is present

The probability that a hazard will be realized is called risk and is assessed as low, medium, high, it is identified by three modes.

2.4.2 Failure modes affect analysis

It is applied to the process and includes systematic listing of each step of the process and then listing every mode of failure of these steps that can affect the quality of the end product.

2.4.3 Fault trees

Fault which may occur in the final product is stated and each process step involved in manufacturing that product is identified with reference to its relevance in causing the stated fault.

2.4.4 Delphi technique

Where a group of experts from different disciplines arrive at a consensus regarding the risk attached to a process or a product. This may be done through a questionnaire circulated to process workers followed by discussion on the answers by the group of experts and possibly more questions and discussion until an informed decision is reached on the risks involved.

2.5 Types of Hazard

2.5.1 Physical hazard

- Hairs
- Stones
- Stems and seeds
- Bones fragments and feathers
- Matchsticks
- Jewellery
- Nails nuts and bolts
- Buttons

- Bidis and cigarettes

2.5.2 Chemical hazards

- Cleaning agents
- Adulterants
- Excess of permissible additive
- Non permissible additive
- Veterinary residue
- Pesticides residue

2.5.3 Biological hazards

2.5.3.1 Invisible

- Bacteria
- Yeast
- Protozoa
- Molds
- Viruses

2.5.3.2 Visible

- Fly
- Worms
- Cockroaches
- Caterpillars
- Weevils

2.6 Principles of HACCP

HACCP is based around seven established principles.

2.6.1 Principle 1

Conduct a hazard analysis: Plants determine the food safety hazards and identify the preventive measures the plant can apply to control these hazards. A food safety hazard is any biological, chemical, or physical property that may cause a food to be unsafe for human consumption.

2.6.2 Principle 2

Identify critical control point: A critical control point (CCP) is a point, step, or procedure in a food process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to an acceptable level.

2.6.3 Principle 3

Establish critical limits for each critical control point: A critical limit is the maximum or minimum value to which a physical, biological, or chemical hazard must be controlled at a critical control point to prevent, eliminate, or reduce to an acceptable level.

2.6.4 Principle 4

Establish critical control point monitoring requirements: Monitoring activities are necessary to ensure that the process is under control at each critical control point. In the United States, the Food Safety and Inspection Service (FSIS) is requiring that each monitoring procedure and its frequency be listed in the HACCP plan.

2.6.5 Principle 5

Establish corrective actions: These are actions to be taken when monitoring indicates a deviation from an established critical limit. The final rule requires a plant's HACCP plan to identify the corrective actions to be taken if a critical limit is not met. Corrective actions are intended to ensure that no product injurious to health or otherwise adulterated as a result of the deviation enters commerce.

2.6.6 Principle 6

Establish record keeping procedures: The HACCP regulation requires that all plants maintain certain documents, including its hazard analysis and written HACCP plan, and records documenting the monitoring of critical control points, critical limits, verification activities, and the handling of processing deviations.

2.6.7 Principle 7

Establish record keeping procedures the HACCP system is working as intended: Validation ensures that the plans do what they were designed to do; that is, they are successful in ensuring the production of safe product. Plants will be required to validate their own HACCP plans.

Verification ensures the HACCP plan is adequate, that is, working as intended. Verification procedures may include such activities as review of HACCP plans, CCP records, critical limits and microbial sampling and analysis. FSIS is requiring that the HACCP plan include verification tasks to be performed by plant personnel. Verification tasks would also be performed by FSIS inspectors. Both FSIS and industry will undertake microbial testing as one of several verification activities.

2.7 Implementation of HACCP

HACCP is a system that assists organizations to identify potential food safety hazards in the entire food supply chain and to take preventive measures for their control. HACCP focuses on the prevention of hazards rather than relying on end product testing. The following sequence of 12 steps, included in the guidelines developed by the Codex Committee on Food Hygiene, is the recommended approach to develop a HACCP programme.

2.7.1 Step 1 Assemble HACCP team

Set up a multi-disciplinary team that includes representatives from production, sanitation, quality control, food microbiology, etc. This team should be assigned specific segments of the food chain to be covered in the HACCP system, and be entrusted with developing a HACCP system as described from Step 2 onwards. Top management must give its full support to the team. If the required expertise is not available within the company, bring in help from a consultant.

2.7.2 Step 2 Describe product

Draw up a full description of the product for which the HACCP plan is to be prepared, including product composition, structure, processing conditions, packaging, storage and distribution conditions, required shelf life, instructions for use, etc.

2.7.3 Step 3 Identify intended use

Identify the intended use of the product by the end-user or consumer. You need to determine where the product will be sold as well as the target group (e.g. institutional catering, homes for senior citizens, hospitals, etc.)

2.7.4 Step 4 Construct flow diagram

You need to carefully examine the product/process and produce a flow diagram around which to base the HACCP study. Whatever the format you choose, study all the steps involved in the process – including delays during or between the steps from receiving the raw material to placing the end-product on the market – in sequence, and present them in a detailed flow diagram with sufficient technical data. In the diagram, you might also want to include the movements of raw materials, products, wastes, a plan of working premises, equipment layout, product storage and distribution, and of employee moves or changes.

2.7.5 Step 5 On-site confirmation of flow diagram

The HACCP team should confirm the processing operation against the flow diagram during all stages and hours of operation and amend the flow diagram if necessary.

2.7.6 Step 6 List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control hazards

Using the flow diagram, the team should list all the hazards – biological, chemical or physical – that may reasonably be expected to occur at each process step, and describe the preventive measures that can be used to control such hazards (for example, the use of air curtains, hand and feet washing at entrance to processing areas, wearing of head gear, use of good manufacturing practices [GMP]/standard operating procedures [SOP]/ sanitation standard operating procedures [SSOP], etc.)

2.7.7 Step 7 Determine critical control points (CCPs)

You may wish to use a decision tree with "yes" or "no" answers to facilitate the determination of CCPs (See Annex A). When applying the decision tree, you need to remain flexible and use common sense to avoid, wherever possible, unnecessary control points throughout the whole manufacturing process. If you identify hazards at a step where control is necessary for safety and no preventive measures exist at that step, you need to modify the process at that step, or at an earlier or a later stage, to include a preventive measure. For example, in a slaughterhouse, covering carcasses with a sanitized cloth to prevent infection by flies is a preventive measure at the carcass stage, which substitutes for a preventive measure such as washing the prepared meat at the next stage, as it will not be possible to disinfect the meat at this stage, i.e., during cutting or mincing operations.

ii) In dairy industry take the case of Paneer which contains as high as 70% moisture which is conducive for microbial growth. Studies carried out on microbial quality of paneer have indicated that it is often contaminated with Staphylococcus aureus and Coliforms. The HACCP has been applied to identify the Critical Control Point for Coliforms and Staphylococcus contamination.The analysis of various samples from raw material to the final product had indicated that the contamination is due to food handlers using bare hands to remove the excess water in paneer (NIN, Hyderabad; Unpublished observations). The food handlers were informed about the importance of personal hygiene and they were asked to wash their hands with soap before touching the paneer, and the quality of paneer was tested after the intervention. Results indicated that cleaning of hands with soap before starting the operation drastically reduced Coliform contamination in the final product.

2.7.8 Step 8 Establish critical limits for each CCP

You need to establish critical limits for each CCP. They are normally derived from specifications included in the food legislation of a country or in national or international standards (e.g. moisture levels in milk powder, or pH level and chlorine limit in potable water, etc.). When limits are not taken from regulatory standards (e.g. frozen storage temperature) or from existing and validated guides of good manufacturing practices, the HACCP team should ascertain the validity of such limits relative to the control of identified hazards and critical points.

2.7.9 Step 9 Establish a system of monitoring each CCP

Monitoring is the scheduled measurement or observation of a CCP to determine conformance to its critical limits. The monitoring procedures must be able to determine loss of control, if any, at the CCP (e.g. improper control of the temperature that may lead to faults in the functioning of a pasteurization unit in a dairy plant). Monitoring for CCPs needs to be done rapidly, as they later relate to on-line processes, and there is usually no time for lengthy analytical testing. Physical and chemical measurements are often preferred as these can be done rapidly and can frequently indicate microbiological control of the product. The programme of observations or measurements should properly identify for each critical point

- Who is to perform monitoring and checking
- When monitoring and checking are performed; and
- How monitoring and checking are performed.

All records and documents associated with monitoring CCPs must be signed by the person(s) doing the monitoring.

2.7.10 Step 10 Establish corrective actions

The HACCP team should develop specific corrective actions and document them in the HACCP plan for each CCP in the HACCP system so that they can deal with deviations when they occur.

Such corrective action should include:

- Proper identification of the person(s) responsible for implementation of a corrective action;
- Actions required to correct the observed deviation;
- Action to be taken with regard to products manufactured during the period when the process was out of control and written records of measures taken.

The actions must ensure, for example, that the CCP has been brought under control, that procedures or conditions that created the out-of-control situation have been corrected, and the food affected, disposed off safely, etc.

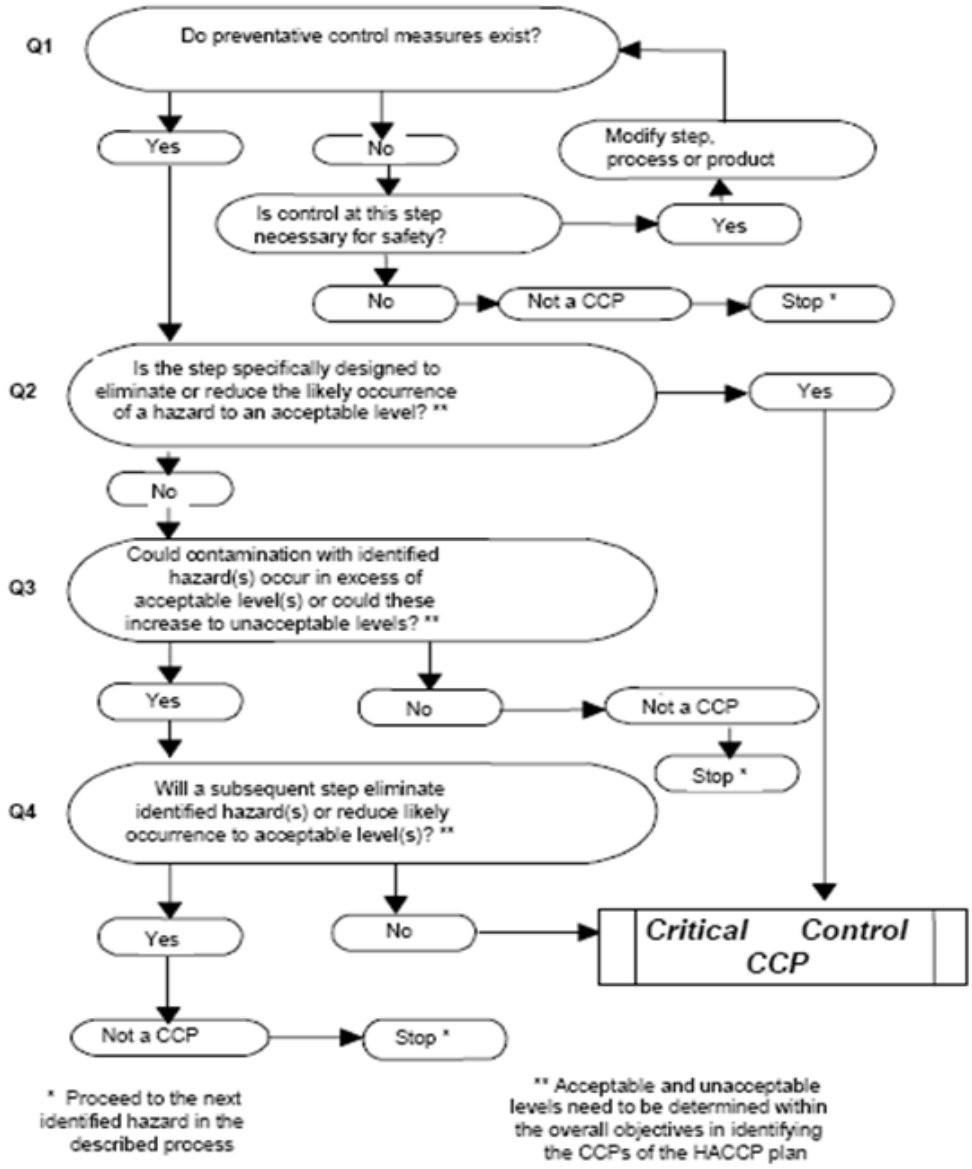
2.7.11 Step 11 Establish verification procedure

Develop a verification procedure to ensure that the HACCP system is working correctly. The procedure should include the frequency of verification, which should be conducted by a responsible and independent person. Examples of verification include auditing methods, random sampling and analysis, etc.

2.7.12 Step 12 Establish documentation and record keeping

The HACCP system requires efficient documentation and accurate record keeping. For example, hazard analysis, identified CCPs and their limits (including revisions, if any) should be documented. Examples of records are CCP monitoring records, records of deviation found and corrective action taken on them, etc.

Annexure- A



EXAMPLE OF DECISION TREE TO IDENTIFY CCPS

Fig. 2.1 Example of decision tree to identify CCPS

2.8 Example of HACCP worksheet

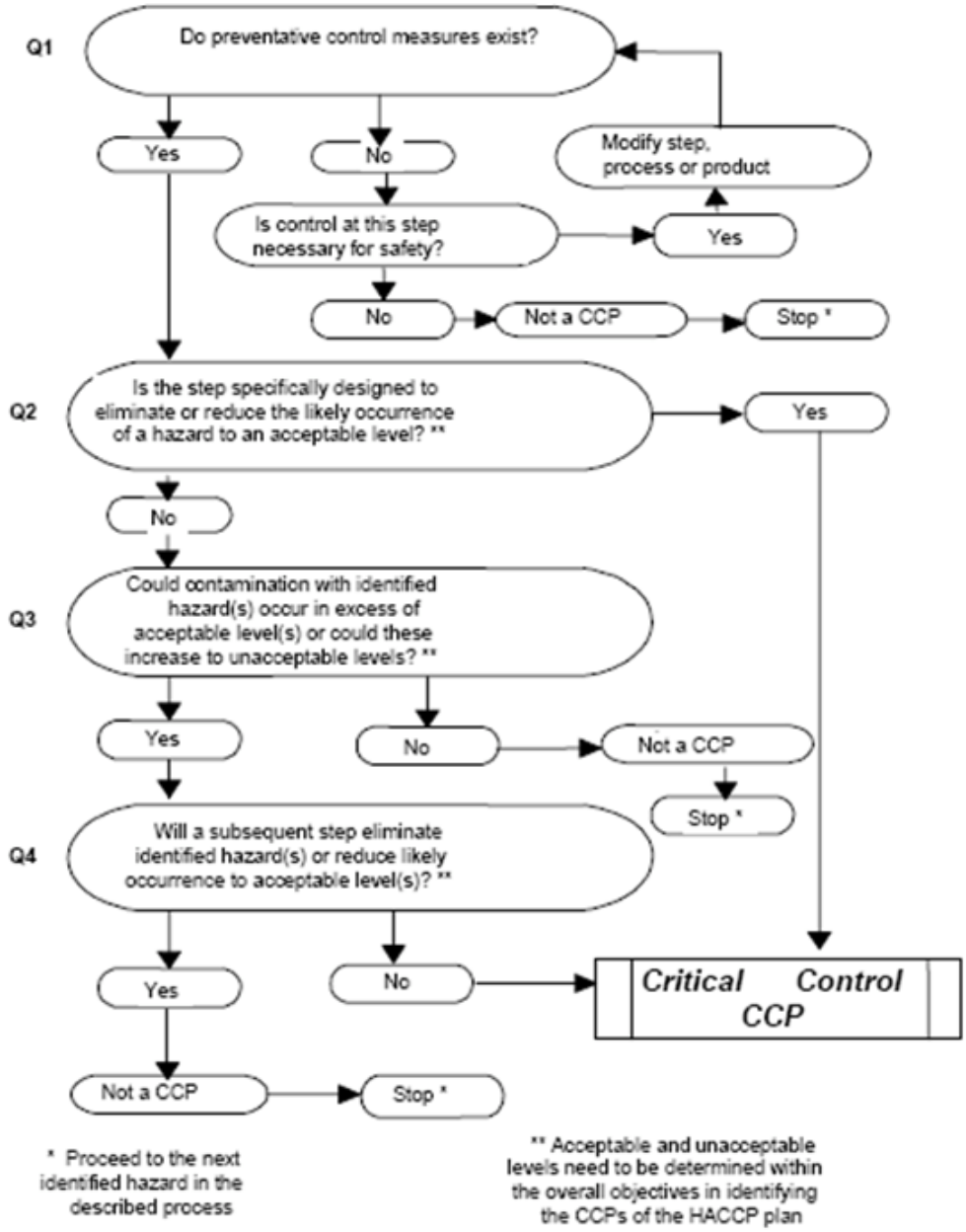


Fig. 2.2 Example of HACCP worksheet

Process steps **will help in identifying the CCPs**

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LESSON 3

NATIONAL REGULATIONS: PFA, AGMARK, BIS and FSSAI

3.1 Introduction

In India, we have mostly two types of standards, which govern the sale of foods including milk and milk products. These are: Legal standards and Quality standards. Moreover, we had many bodies enforcing standards. This led to lot of confusion for both manufacturers as well as enforcers. In the recent past the concept of globalization or flat word has emerged very strongly, where all the boundaries have been eliminated. However, some barriers to trade with respect to more stringent norms and others have been raised. In this context Indian Standards needed to be harmonized within themselves as well as with the international standards. To eliminate the problems and confusions in the trade of food items including milk, a thought of uniform standards was evolved, which has been culminated in the form of Food Safety and Standards Authority of India.

3.2 Legal Standards

Legal standard means the specifications or the requirements which pertain to the law of the Govt. and are set up by the Govt. to meet certain minimum requirements in terms of chemical quality (i.e. composition), bacteriological quality (i.e. hygienic quality), and labeling and packaging requirements.

In our country, legal standards are given under PFA Rules, 1955 (which are amended from time to time). Legal standards or PFA standards prescribe the minimum requirements for all types and categories of food. These standards are consistent with the minimum quality that is attainable under Indian conditions by the majority of the farmers, producers, processors, sale agencies etc. Further, any food that does not confirm to the minimum standards laid down by the legal rulers (PFA rules) is said to be adulterated, irrespective whether anything has been added to or removed from the original food.

3.3 Parameters Generally Taken into Consideration while Fixing Legal Standards

3.3.1 Purity

There should be a mention of purity in clear terms i.e. whether anything can be added or removed from the original food should be clearly specified in the rules.

3.3.2 Composition

Certain compositional criteria should also be clearly specified in the rules, as to what should be the minimum level of components in a given type of product. For example, fat and solids-not-fat content in milk, fat in whole milk powder, cream, paneer etc. Similarly, moisture in ghee, butter, skim milk powder etc. Likewise, all the compositional requirements should be specified for all the dairy products.

3.3.3 Additives

If any additives are required to be added to any food to improve its quality, stability, flavour etc, these should be clearly specified in the rules with respect to their levels etc. For example, certain additives are permitted like nisin (preservative) in cheese, BHA (antioxidant) in ghee, butter, whole milk powder etc.

3.3.4 Efficiency of processing

It should be clearly specified in the rules as to which types of tests have to be performed to check the efficiency of heat processing treatments given to milk and milk products. For example, phosphatase test should be negative for pasteurized milk; turbidity test should be negative for sterilized milk.

3.3.5 Bacteriological quality (Hygienic quality)

Permissible limits for coliform count, total count, yeast and mould count etc should be clearly specified in the rules

3.3.6 Packaging and labeling requirements

There should be mention in the rules as to what type of packaging material is to be used for what type of food and what should be indicated on the labels like quantity, price, manufacturing date, expiry date etc. Now- a- days, nutritional facts are to be given on the labels.

Fig. 3.1 BIS Mark and AGMARK

3.4 Quality standards

Quality standards means those specifications which are laid down by the Govt or some expert body constituted by the Govt. for the purpose of producing high quality products.

While legal standards are compulsory, the quality standards are not compulsory. They are on voluntary basis.
In our country, we have two types of quality standards

- BIS/ ISI standards
- Agmark standards

Both of these are above the PFA minimum standards. Both of these are useful for producing export quality products.

BIS/ISI standards deal with many types of processed food products, apart from non-food products. Similarly, Agmark standards deal with many types of foods, mainly with the raw agricultural produce. For example: cereals, oils, oil seeds, spices, eggs, legumes (pulses), ghee, butter etc.

Among the dairy products, for the purpose of quality standards, ghee and creamery butter (Table butter or salted butter) are covered under Agmark. Rest of the dairy products are covered under BIS/ISI.

As stated earlier, Agmark and BIS are voluntary and not compulsory; but in 1987, BIS has made it compulsory or mandatory for certain items to have ISI mark. For example, for food colors and additives, vanaspati, containers for packing, milk powder and condensed milk. Therefore, for these two milk products (milk powder and condensed milk), it is now compulsory to have ISI mark.

3.5 Why Do We Need Legal and Quality Standards?

The main purpose of these standards is to protect the interest of the consumer, although in a way the interest of the manufacturer also gets protected, because if it is a certified product with some quality mark (like ISI, Agmark), it will sell more as compared to the uncertified product.

Moreover, customer also wants to be sure about quality. He does not mind to pay a little more for an assumed quality product i.e. certified product.

Furthermore, someone has to protect the interest of the consumer. They should not be left at the mercy of the manufacturers, because he is ignorant about the quality. Consumer should get a product of pure quality (i.e. unadulterated) free from pathogenic organisms and also free from harmful substances like pesticides, antibiotics, heavy metals (toxic metals like Arsenics, lead, mercury etc), and toxins etc.

So, it is the duty of the Govt. to fix legal standards to protect the interest of the consumer and also to fix quality standards so as to improve the quality of the product to a higher degree above the minimum legal (PFA) standards.

3.6 PFA Act and Rules

PFA stands for Prevention of Food Adulteration. The PFA Act was passed in 1954 and PFA Rules were framed in 1955 to protect the consumers against the supply of inferior quality or adulterated food. In recent years the Govt. of India has enacted another Act known as "The Food Safety and Standards Act, 2006", abbreviated as FSS Act 2006. The regulations under this act have come into force from Aug, 2011.

The main objectives of PFA Act are:

- To protect the public from harmful and poisonous foods.
- To prevent the sale of substandard food containing harmful substances, and
- To protect the society against unscrupulous and anti-social dealers by eliminating fraudulent practices.

PFA standards are formulated and revised by an expert body called Central Committee for Food standards (CCFS) under the Directorate General of health Services, Ministry of Health and Family Welfare. It is the CCFS which advices the Central Govt. and the State Govt. on matters arising out of the administration of PFA Act. It is a very heavy committee .People from all the States and the Union Territories (UTs) and all the major Ministries and departments are representative of this committee.

3.7 Procedure for collection and analysis of PFA samples

PFA samples are collected by Food Inspectors. After collecting the samples, he divides the sample then and there into 3 parts. One part is sent for analysis to Public Analyst (under the control of local health authority, usually the chief medical officer (CMO). Two parts are given to local health authority (LHA) for custody.

The public analyst has to send the report of analysis within 40 days of receipt of sample. In case of adverse report of public analyst, the 2nd part of sample is produced in the court within 7 days and the copy of report is given to party i.e. accused by the local health authority (CMO). Within a period of 10days of the report, LHA or party concerned or both of them may make an application to the court for getting the 2nd part of the sample analyzed at Central Food Laboratory (CFL).

On receipt of such report, the court sends the 2nd part of sample for analysis to Director, CFL, who has to send a certificate on the result of analysis within one month from the receipt of 2nd part of sample.

The 3rd part of the sample is kept to meet such exigencies like damage /destruction / breakage on the way when first part of sample is sent to Public Analyst for analysis.
The report of public analyst on 1st part of the sample stands superseded by the certificate issued by the Director, CFL on analysis of the 2nd part of the sample.
There are several Public Health Laboratories (also called Public Food Laboratories) in the country where first part of the sample is analysed. Almost each district has such a lab under the control of Chief Medical Officer (called Local Health Authority).

But there are only four CFL (central food laboratories) in the country. These are located at Kolkata, Mysore, Pune and Ghaziabad. All these 4 CFLs take care of the requirements of whole country, Zone wise. In fact the whole country is divided into 4 Zones and each zone is then connected to one of these CFL. Samples of a particular area/zone are sent to the concerned CFL.

3.8 Preservative Permitted To Be Added To Samples

When a food inspector takes the sample of any food for analysis, he has to add a preservative, as may be prescribed from time to time, so as to keep the sample in a condition suitable for analysis. The preservative used in the case of samples of any milk (including toned, separated and skimmed milk, standardized milk chhanna, skimmed milk chhanna, cream, ice-candy, dahi, khoa or khoa based or paneer based sweets, such as kalakand and burfi, chutney and prepared foods, gur, coffee and tea in liquid and semi liquid form, shall be the liquid commonly known as "formalin", a liquid containing about 40 percent of formaldehyde in aqueous solution in the proportion of 0.1 ml (two drops) for 25ml or 25grams (i.e.@ 0.4%). Provided that in case of ice-cream and mixed ice-cream, the preservative used shall be in the proportion of 0.6 ml for 100 ml or 100 gm (i.e.@ 0.6%).

3.9 Agriculture Produce (Grading and Marking) Act (AGMARK)

Agmark stands for "Agricultural Marking". In order to have a systematic marketing of Agricultural Produce on the basis of well defined quality, Indian Legislature in 1937 passed an act known as "Agriculture Produce (Grading and Marking) Act, 1937. This act is not mandatory. It is permissive in nature. It is one's choice to go for Agmark grading, if one can meet their specifications. Rules under this Act are called "General Grading and Marking Rules, 1937". Rules have been revised in 1988 and are called General Grading & Marking Rules, 1988. Grading of Agricultural items under these rules is called AGMARK GRADING or Agmark certification.

Agmark is the exclusive property of Govt. of India. It is not a private trade mark. Directorate of Marketing & Inspection (DMI) is the authority on the Agmark whose head quarter is now at Faridabad and branched head quarter is at Nagpur. It is the DMI which enforces the Agricultural Produce Act, 1937.

Under the Agricultural Produce Act, 1937-grade standards are given for agricultural and allied commodities like cereals, oil seeds, oils, creamery butter, ghee, legumes, eggs etc. Agricultural commodities are categorized into various grades such as, special, good, fair, ordinary etc depending upon the degree of quality (type of composition) in each case. These grades are known as "Agmark Standards" and this way of categorizing or grading the agricultural products in terms of their chemical composition or quality is called "Agmark Grading".

3.10 Objectives of Agmark Scheme

- To assure the consumers a product of pre-tested quality & purity.

- To enable the producer of good quality products to have better returns.

- To have a sale of the product in the market with a uniform composition and well defined quality.

- To eliminate the malpractice of adulteration in the movement of the product from producer to consumer.

As told earlier, among the dairy products, only ghee and creamery butter (Table butter or salted butter) are graded under Agmark. Deshi or cooking (unsalted or white) butter is not graded under Agmark.

3.11 How The Certificate of The Authority To Use Agmark Labels Is Obtained?

Interested parties who wish to get authority to use Agmark Labels have to apply to the Agricultural Marketing Advisor (AMA) to Govt. of India at Directorate of Marketing & Inspection (DMI) whose H.Q is at Faridabad (Branched H.Q. at Nagpur). Application by the party concerned should be submitted through the state marketing officer.

Interested parties should meet the following pre-requisites so as to get the authority to use Agmark labels.

- They should have well equipped, hygienic site and hygienic equipments.

• They should have well equipped lab.

• They should have qualified staff like butter makers having 5 years experience or possessing a certificate of proficiency from a recognized agricultural or dairy institute. Similarly, a chemist with dairying degree in dairying.

• Packers of pasteurized table butter should have pasteurizing plant and cold storage facilities.

• Cream separating station, if separate, that also should be hygienic.

• They should have quality control checks on cream and raw materials so as to ensure that no adulteration with animal or vegetable fat takes place.

• Packing containers should be clean and rust free.

• No vegetable fat, animal body fat and no artificial flavoring or coloring matter should be seen near the factory and cream separating station.

• Ghee clarification temperature should never go above 110°C.

• They should also send one sample of butter/ghee to Regional Agmark Lab or any other specified lab at regular intervals as advised by AMA.

• All instructions from AMA will have to be followed strictly. For example, method of sampling, method of sealing and marking of tins or cartons, maintenance of records, labeling procedure as prescribed by AMA. Upto date record of labels to be kept, labels to indicate clearly the designation (pasteurized butter, special & general grade of ghee etc) and serial number of label. Container should indicate the name of packer, batch no. (Melt no), date of packing, net weight and Certificate of Authorization (CA) number.

Now if parties concerned can afford, then write to AMA at Faridabad/Nagpur through state marketing officer. Then, AMA or his authorized persons will inspect the premises, the facilities and technical staff etc. After satisfaction, AMA issues the necessary certificate of authority to use Agmark Labels. Certificate of Authorization is renewed periodically on the basis of the past performance of the authorized packer.

3.12 How The Agmark Scheme Operates In Case Of Butter & Ghee?

Once the authority to use Agmark Label for butter/ghee is obtained, then usually the Agmark people employ their chemist (called Agmark chemist) in the factory.

All the operations right from the stage of manufacturing the product to the stage of packing and sealing are done under the supervision of Agmark chemist.

Usually the butter, ghee etc are manufactured in the factory itself, but sometimes the Kacha/Raw ghee is brought to the factory by middlemen, or agents or producers, then the Agmark chemist draws sample of that Raw or Kacha ghee and analyses for BR reading, FFA, Baudouin test, RM and Polenske value etc for his satisfaction about its purity, before its further processing.

After the manufacturing of every batch of butter or ghee, but before the packing and sealing, the product is tested for purity and quality by the Agmark chemist as per the Agmark standard of the concerned product.

Agmark chemist draws two samples of butter, one is analysed and second is sent to Regional Agmark Laboratory as specified by AMA. Similarly in case of ghee, he draws three samples, one is analysed and second is sent to central control lab as per AMA and 3rd is given to manufacturer for future use.

Once the Agmark chemist is satisfied with the analysis with regard to the specifications fixed under Agmark, then only labels are issued and fixed, otherwise it is denied.

Fixing of labels is done by a special adhesive in such a manner that once fixed cannot be removed without damage.

In case the Agmark chemist is not employed, then the authorized packer can get his product tested at the approved State Grading Laboratory (SGL).

After the ghee and butter are packed and sealed in tins/cartons and sold in the market, the field staff of DMI collects the check samples from the market when the products are on sale in the market. These check samples are analysed at different Regional Agmark Laboratories (RAL) and the results are compared with the original. If the results agree then alright, but if the results do not agree, then appropriate action is taken, such as the product is withdrawn from the market, and the authority to use Agmark labels is cancelled etc.

3.13 Bureau of Indian Standards (B.I.S): It is a national standards body of India which is responsible for formulating National Standards for various types of articles (both edible & non-edible i.e. food & non-food articles e.g. live stock feed, cattle housing, equipments, dairy products, food additives, food hygiene), testing apparatus and methods etc.
The old name of this organization was ISI (*Indian Standards Institution*), which was established in 1947. The new name i.e. BIS came into existence from 1st April, 1987 under the BIS Act 1986.

Structure of BIS/ Members of BIS

Membership of BIS is broad based and all important interests are represented.
Minister for food and civil supplies is the *President* of BIS

Members of BIS include:

- Members of Parliament,
- Ministers of state govts.,
- Nominees of central Govt. Ministries and departments,
- Farmers community,
- Consumers organizations,
- Academic institutions,
- Research institutions,
- Industry and
- Professional Associations.

OBJECTIVES and FUNCTIOS OF BIS

1. To formulate Indian standards for various articles, processes, methods of test, codes of practices etc and promote their implementation.
2. To promote the Concepts of standardization and Quality control in industries.

3. To coordinate the efforts of producers and users for making improvements in the materials, products, processes and methods.
4. To operate ISI certification scheme.
5. To establish testing laboratories of its own.
6. To operate laboratory recognition scheme to meet the requirements of testing.
7. To offer technical and consultancy services within and outside the country.
8. To have cooperation and coordination with international standard making bodies like ISO.

BIS is a member of ISO and IEC

ISO- international organization for standardization.
IEC- international electro-technical commission.

How to get authority to use ISI mark?

For getting an authority/or license to use ISI mark on the products, the manufactures have to apply to BIS for permission.
BIS people then send a team to inspect the factory, verify the capabilities of manufacturer to produce and also test the products on continuous basis in accordance with the relevant Indian standards.
They also check the quality of the products and other facilities and after satisfying themselves they give permission to use ISI mark.
BIS gives a well defined quality control system to the manufactures and tells them to exercise control measures at various stages like,

- Raw material,
- Diff. stages of production,
- Finished product stage.

BIS keeps a supervisory control to ensure that the ISI marks product are always in conformity with the relevant Indian standards.

BIS also has an elaborate Quality Audit System under which they draw the samples

- From the factory production line, or
- From factory store , or
- Purchase them from the open market.

And then test the samples in their own labs or recognized labs to check the conformity with the relevant Indian standards.

BIS also entertains complaints from consumers and arranges free replacements of defective ISI-marked goods.

BIS also provides training in statistical quality control to its applicants and licensees for improving their technical skill.

3.14 Modern Integrated Food Law (FSSA, 2006)

In order to consolidate the laws relating to food and to establish the Food Safety and Standards Authority of India for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food for human consumption and for matters connected therewith or incidental thereto, the Govt. of India has enacted new food laws known as "The Food Safety and Standards Act, 2006". This Act was passed on 23rd August, 2006. It extends to the whole of India.

However, the Act came in to force only recently in 2011. This Act consolidates various acts & orders that have hitherto handled food related issues in various Ministries and Departments.

3.14.1 Highlights of the Food Safety and Standard Act, 2006

Various central Acts like Prevention of Food Adulteration Act, 1954 , Fruit Products Order , 1955, Meat Food Products Order , 1973, Vegetable Oil Products (Control) Order, 1947, Edible Oils Packaging (Regulation) Order 1988, Solvent Extracted Oil, De- Oiled Meal and Edible Flour (Control) Order, 1967, Milk and Milk Products Order, 1992 etc will treated as repealed after commencement of FSS Act, 2006.

The Act also aims to establish a single reference point for all matters relating to food safety and standards, by moving from multi- level, multi- departmental control to a single line of command. To this effect, the Act establishes an independent statutory Authority – the Food Safety and Standards Authority of India with head office at Delhi. Food Safety and Standards Authority of India (FSSAI) and the State Food Safety Authorities shall enforce various provisions of the Act.

3.14.2 Establishment of FSSAI

Ministry of Health & Family Welfare, Government of India is the Administrative Ministry for the implementation of FSSAI. The Chairperson and Chief Executive Officer of Food Safety and Standards Authority of India (FSSAI) have already been appointed by Government of India. The Chairperson is in the rank of Secretary to Government of India. FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption.

3.14.3 Composition of Food Safety and Standards Authority of India

The Food Authority consists of a Chairperson and the following twenty-two members out of which one-third shall be women, namely:-

(a) Seven Members, not below the rank of a Joint Secretary to the Government of India, to be appointed by the Central Government, to respectively represent the Ministries or Departments of the Central Government dealing with -

(i) Agriculture, (ii) Commerce, (iii) Consumer Affairs, (iv) Food Processing, (v) Health, (vi) Legislative Affairs, (vii) Small Scale Industries, who shall be Members *ex-officio*;

(b) Two representatives from food industry of which one shall be from small scale industries;

(c) Two representatives from consumer organizations.

(d) Three eminent food technologists or scientists.

(e) Five members to be appointed by rotation every three years, one each in seriatim from the Zones as specified in the First Schedule to represent the States and the Union territories;

(f) Two persons to represent farmers' organizations.

(g) One person to represent retailers' organizations.

3.14.4 Punishment for unsafe food under FSSAI

Any person who, whether by himself or by any other person on his behalf, manufactures for sale or stores or sells or distributes or imports any article of food for human consumption which is unsafe, shall be punishable,–

(i) where such failure or contravention does not result in injury, with imprisonment for a term which may extend to six months and also with fine which may extend to one lakh rupees;

(ii) where such failure or contravention results in a non-grievous injury, with imprisonment for a term which may extend to one year and also with fine which may extend to three lakh rupees;

(iii) where such failure or contravention results in a grievous injury, with imprisonment for a term which may extend to six years and also with fine which may extend to five lakh rupees;

(iv) where such failure or contravention results in death, with imprisonment for a term which shall not be less than seven years but which may extend to imprisonment for life and also with fine which shall not be less than ten lakh Rupees.

3.14.5 Penalty for selling food not of the nature or substance or quality demanded

Any person who sells to the purchaser's prejudice any food which is not in compliance with the provisions of this Act or the regulations made there under, or of the nature or substance or quality demanded by the purchaser, shall be liable to a penalty not exceeding five lakh rupees. Provided that the persons covered under sub-section (2) of section 31, shall for such non-compliance be liable to a penalty not exceeding twenty five thousand rupees.

3.14.6 Penalty for sub-standard food

Any person who whether by himself or by any other person on his behalf manufactures for sale or stores or sells or distributes or imports any article of food for human consumption which is sub-standard, shall be liable to a penalty which may extend to five lakh rupees.

3.13.7 Penalty for possessing adulterant

(1) Subject to the provisions of this chapter, if any person who whether by himself or by any other person on his behalf, imports or manufactures for sale, or stores, sells or distribute any adulterant shall be liable –

(i) where such adulterant is not injurious to health, to a penalty not exceeding two lakh rupees;

(ii) where such adulterant is injurious to health, to a penalty not exceeding ten lakh rupees.

(2) In a proceeding under sub-section (1), it shall not be a defence that the accused was holding such adulterant on behalf of any other person.

As per new norms the Milk and Milk Products Order, 1992 shall be deemed to be regulations made under this Act.

(1) On and from the date of commencement of this Act, the Milk and Milk Products Order, 1992 issued under the Essential commodities Act, 1955 (10 of 1955) shall be deemed to be the Milk and Milk Products Regulations, 1992 issued by the Food Authority under this Act.

(2) The Food Authority may, with the previous approval of the Central Government and after previous publication, by notification, amend the regulations specified in sub-section.

In FSSAI, to harmonise the codex standards, **"Codex India"** the National Codex Contact Point (NCCP) for India, has been constituted. This point is located at Food Safety and Standards Authority of India (Ministry of Health and Family Welfare), FDA Bhawan, Kotla Road, New Delhi -110002, India. It coordinates and promotes Codex activities in India in association with the National Codex Committee and facilitates India's input to the work of Codex through an established consultation process.

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LESSON 4
INTERNATIONAL REGULATIONS: CODEX, IDF, ISO, FDA, EEC

4.1 Introduction

The different sets of standards arising from the spontaneous and independent development of food laws and standards by different countries inevitably gave rise to trade barriers that were of increasing concern to food traders in the early twentieth century. Trade associations that were formed as a reaction to such barriers pressured governments to harmonize their various food standards so as to facilitate trade in safe foods of a defined quality. The International Dairy Federation (IDF), founded in 1903, was one such association. Its work on standards for milk and milk products later provided a catalyst in the establishment of the Codex Alimentarius Commission and in the setting of its procedures for elaborating standards.

4.2 Codex Alimentarius Commission (CAC)

The FAO and the WHO jointly established the Codex Alimentarius Commission (CAC) in 1962 to implement the joint FAO/WHO food Standards Programme. The aim of the commission is to protect the health of consumers by ensuring observance of fair practices in food trade. It promotes co-ordination of work on formulation of food standards undertaken by international governmental and non-governmental organizations. The Codex Alimentarius Commission shall be responsible for making proposals to, and shall be consulted by, the Directors-General of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) on all matters pertaining to the implementation of the joint FAO/WHO Food Standards Programme. In addition to commodity standards, the Codex Alimentarius includes general standards, which have across-the-board application to all foods and are not product-specific. There are general standards or recommendations for:

- Food labeling;
- Food additives;
- Contaminants;
- Methods of analysis and sampling;
- Food hygiene;
- Nutrition and foods for special dietary uses;
- Food import and export inspection and certification systems;
- Residues of veterinary drugs in foods;
- Pesticide residues in foods.

Most standards take a number of years to develop. Once adopted by the Commission, a Codex standard is added to the Codex Alimentarius. The Codex Alimentarius now has such a well-established reputation as an international reference that it has become customary for health authorities, government food control officials, manufacturers, scientists and consumer advocates to ask first of all: What does the Codex Alimentarius have to say? Adoption of HACCP standards, formulated by CAC, under the sanitary and phytosanitary (SPC) measures has made the HACCP system an instrument of food safety. It has become incumbent on signatory countries of the SPC agreement to implement these standards.

4.3 International Dairy Federation (IDF)

IDF is the pre-eminent source of scientific and technical expertise for all stakeholders of the dairy chain. Membership covers 56 countries and is growing. The mission of IDF is to represent the dairy sector worldwide by providing the best global source of scientific expertise and knowledge in support of the development and promotion of quality milk and dairy products to deliver consumers with nutrition, health and well-being.

IDF publishes **jointly with the ISO**, standards method of sampling and analysis of milk and milk products.

4.3.1 Work Areas of IDF

Fig. 4.1 Areas of IDF

4.4 International Organization for Standardization (ISO)

ISO is a network of the national standards institutes of 158 countries, one member per country, with a Central Secretariat in Geneva, Switzerland, that coordinates the system. ISO is a non-governmental organization that forms a bridge between the public and private sectors. It is the world's largest developer and publisher of International Standards. ISO enables a consensus to be reached on solutions that meet both the requirements of business and the broader needs of society.

"International Organization for Standardization" would have different acronyms in different languages e.g. "IOS" in English, "OIN" in French (Organisation internationale de normalisation).

Its founders decided to give it also a short, all-purpose name. They chose "ISO", derived from the Greek isos, meaning "equal". Whatever the country, whatever the language, the short form of the organization's name is always ISO.

4.4.1 Characteristics of ISO standards are:

4.4.1.1 Democratic

Every full member of ISO has the right to take part in the development of any standard which it judges to be important to its country's economy. No matter what the size or strength of that

economy, each participating member in ISO has one vote. Each country is on an **equal footing** to influence the direction of ISO's work at the strategic level, as well as the technical content of its individual standards.

4.4.1.2 Voluntary

ISO standards are voluntary. As a non-governmental organization, ISO has no legal authority to enforce the implementation of its standards. ISO does not regulate or legislate. However, countries may decide to adopt ISO standards - mainly those concerned with health, safety or the environment - as regulations or refer to them in legislation, for which they provide the technical basis. In addition, although ISO standards are voluntary, they may become a **market requirement**, as has happened in the case of ISO 9001 quality management systems, or of dimensions of freight containers and bank cards. ISO itself does not regulate or legislate.

4.4.1.3 Market-driven

ISO only develops standards for which there is a **market requirement**. The work is mainly carried out by experts from the industrial, technical and business sectors which have asked for the standards, and which subsequently put them to use.

4.4.1.4 By Consensus

ISO standards are based on international consensus among the experts in the field. Consensus, like technology, evolves and ISO takes account both of evolving technology and of evolving interests by requiring a **periodic review** of its standards at least every five years to decide whether they should be maintained, updated or withdrawn. In this way, ISO standards retain their position as the **state of the art**.

4.4.1.5 Globally relevant

ISO standards are technical agreements which provide the framework for c**ompatible technology worldwide**. They are designed to be globally relevant - useful everywhere in the world. ISO standards are useful everywhere in the world.

4.5 Food and Drug Administration (FDA or USFDA)

FDA is an agency of the United States Department of Health and Human Services. The FDA is responsible for protecting and promoting public health through the regulation and supervision of food safety, tobacco products, dietary supplements, prescription and over-the-counter pharmaceutical drugs (medications), vaccines, biopharmaceuticals, blood transfusions, medical devices, electromagnetic radiation emitting devices (ERED), veterinary products, and cosmetics.

The FDA also enforces other laws, which include sanitation requirements on interstate travel and control of disease on products ranging from certain household pets to sperm donation for assisted reproduction.

The FDA is formed in 1906 and has its headquarters at White Oak, Maryland. The agency also has 223 field offices and 13 laboratories located throughout the 50 states. In 2008, the FDA started opening offices in foreign countries, including China, India, Costa Rica, Chile, Belgium, and the United Kingdom.

4.5.1 Organizations of FDA

The FDA comprises several offices and centers. Major are

- Center for Biologics Evaluation and Research
- Center for Devices and Radiological Health (CDRH)
- Center for Drug Evaluation and Research (CDER)
- Division of Manufacturing and Product Quality
- Division of New Drugs and Labeling Compliance
- Division of Scientific Investigations
- Division of Drug Marketing, Advertising and Communications8
- Informatics and Computational Safety Analysis Staff (ICSAS)
- Center for Food Safety and Applied Nutrition
- Center for Tobacco Products
- Center for Veterinary Medicine
- National Center for Toxicological Research
- Office of Regulatory Affairs

4.5.2 What does FDA regulates

The FDA regulates more than $1 trillion worth of consumer goods, about 25% of consumer expenditures in the United States. This includes $466 billion in food sales, $275 billion in drugs, $60 billion in cosmetics and $18 billion in vitamin supplements. Much of the expenditures are for goods imported into the United States; the FDA is responsible for monitoring a third of all imports. Most federal laws concerning the FDA are part of the Food, Drug and Cosmetic Act.

4.5.3 Regulatory programs of FDA

The programs for safety regulation vary widely by the type of product, its potential risks, and the regulatory powers granted to the agency. For example, the FDA regulates almost every facet of prescription drugs, including testing, manufacturing, labelling, advertising, marketing, efficacy and safety, yet FDA regulation of cosmetics is focused primarily on labelling and safety. The FDA regulates most products with a set of published standards enforced by a modest number of facility inspections. FDA regulates in the following areas:

4.5.3.1 Food and dietary supplements

The Center for Food Safety and Applied Nutrition (CFSAN) is the branch of the FDA that is responsible for ensuring the safety and accurate labelling of nearly all food products in the United States. One exception is meat products derived from traditional domesticated animals, such as cattle and chickens, which fall under the jurisdiction of the United States Department of Agriculture Food Safety and Inspection Service.

CFSAN's activities include establishing and maintaining food standards, such as standards of identity (for example, what the requirements are for a product to be labelled, "yogurt") and standards of maximum acceptable contamination. CFSAN also sets the requirements for nutrition labelling of most foods. Both food standards and nutrition labelling requirements are part of the Code of Federal Regulations. Bottled water is regulated in America by the FDA. State governments also regulate bottled water. Tap water is regulated by state and local regulations, as well as the United States Environmental Protection Agency (EPA).

4.5.3.2 Drugs

The Centre for Drug Evaluation and Research has different requirements for the three main types of drug products: new drugs, generic drugs and over-the-counter drugs. A drug is considered "new" if it is made by a different manufacturer, uses different excipients or inactive ingredients, is used for a different purpose, or undergoes any substantial change.

4.5.3.3 Vaccines, blood and tissue products, and biotechnology

The Centre for Biologics Evaluation and Research is the branch of the FDA responsible for ensuring the safety and efficacy of biological therapeutic agents. These include blood and blood products, vaccines, allergenics, cell and tissue-based products, and gene therapy products. The original authority for government regulation of biological products was established by the 1902 Biologics Control Act, with additional authority established by the 1944 Public Health Service Act. Along with these Acts, the Federal Food, Drug, and Cosmetic Act applies to all biologic products, as well. Originally, the entity responsible for regulation of biological products resided under the National Institutes of Health; this authority was transferred to the FDA in 1972.

4.5.3.4 Medical and radiation-emitting devices

The Centre for Devices and Radiological Health (CDRH) is the branch of the FDA responsible for the premarket approval of all medical devices, as well as overseeing the manufacturing, performance and safety of these devices. A medical device includes products from the simple toothbrush to complex devices such as implantable brain pacemakers. CDRH also oversees the safety performance of non-medical devices that emit certain types of electromagnetic radiation. Examples of CDRH-regulated devices include cellular phones, airport baggage screening equipment, television receivers, microwave ovens, tanning booths, and laser products.

4.5.3.5 Cosmetics

Cosmetics are regulated by the Centre for Food Safety and Applied Nutrition, the same branch of the FDA that regulates food. Cosmetic products are not in general subject to premarket approval by the FDA unless they make "structure or function claims", which make them into drugs. However, all color additives must be specifically approved by the FDA before they can be included in cosmetic products sold in the U.S. The labelling of cosmetics is regulated by the FDA, and cosmetics that have not been subjected to thorough safety testing must bear a warning to that effect.

4.5.3.6 Veterinary products

The Centre for Veterinary Medicine (CVM) is the branch of the FDA that regulates food, food additives, and drugs that are given to animals, including food animals and pets. CVM does not regulate vaccines for animals; these are handled by the United States Department of Agriculture. CVM's primary focus is on medications that are used in food animals and ensuring that they do not affect the human food supply. The FDA's requirements to prevent the spread of bovine spongiform encephalopathy are also administered by CVM through inspections of feed manufacturers.

4.5.3.7 Tobacco products

Since the Family Smoking Prevention and Tobacco Control Act became law in 2009, the FDA also has had the authority to regulate tobacco products. In 2009, Congress passed a law requiring color

warnings on cigarette packages and on printed advertising, in addition to text warnings from the U.S. Surgeon General. The nine new graphic warning labels were announced by the FDA in June 2011 and are required to appear on packaging by September 2012.

4.6 What doesn't FDA regulate?

FDA does not regulate:

- advertising (except for prescription drugs, medical devices, and tobacco products)
- alcoholic beverages
- some consumer products, such as paint, child-resistant packages, baby toys, and household appliances (except for those that give off radiation)
- illegal drugs of abuse, such as heroin and marijuana
- health insurance
- meat and poultry (except for game meats, such as venison, ostrich, and snake)
- restaurants and grocery stores

FDA shares the responsibility for regulating these products with other government agencies:

pesticides (FDA, the U.S. Department of Agriculture, and the Environmental Protection Agency regulate these)

4.7 FDA and India

FDA's activities in India are to ensure that food and medical products exported from India to the U.S. are safe, are good quality, and are effective; these efforts include obtaining better and more robust information to help FDA officials in the various FDA headquarter Offices and centers and at the borders make better decisions about products from India that are being developed for the U.S. market. This includes products being reviewed for marketing authorization in the U.S., and that are already on the U.S. market.

To this end, FDA activities in India include:

- Engaging with Indian counterpart regulatory authorities to ensure the timely exchange of information regarding clinical trials that are conducted that support marketing applications in the U.S.

- Partnering with Indian counterpart agencies on various bilateral and regional capacity building initiatives

- Working with regulated product industries in India that wish to export their products to the U.S. to assure their understanding of our standards and expectations regarding FDA-regulated products

- Coordinating and collaborating daily on product quality and safety issues with other U.S. government agencies that have complementary missions to assess conditions and events in those areas that might have an impact on the safety and quality of FDA-regulated products being exported to the U.S.

- Increased FDA inspections of relevant high-risk facilities; and by working with private- and

public-sector entities that wish to engage with FDA on third-party certification efforts regarding these products.

The **European Economic Community (EEC)** (sometimes simply known as the **European Community**, also known as the **Common Market** in the English-speaking world) was an international organisation created with a view to bring about economic integration (including a common market) among its six original members—Belgium, France, Germany, Italy, Luxembourg and the Netherlands.

4.8 Aims and achievements

The main aim of the EEC, as stated in its preamble, was to "preserve peace and liberty and to lay the foundations of an ever closer union among the peoples of Europe". Calling for balanced economic growth, this was to be accomplished through:

- The establishment of a customs union with a common external tariff
- Common policies for agriculture transport and trade
- Enlargement of the EEC to the rest of Europe

For the customs union, the treaty provided for a 10% reduction in custom duties and up to 20% of global import quotas.

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Lesson 5

TERMINOLOGY OF STANDARD SOLUTIONS AND REAGENTS

5.1 Introduction

Standard solutions and reagents are the core of any chemical analysis. The accuracy, repeatability and precision of chemical analysis of food or dairy products depend upon the accuracy of the reagents and solutions used in the analysis. Therefore, it is very important that the reagents and the solutions should have the correct strength and should be checked from time to time before use. To express the concentration or strength of solutions and reagents different terms are used in the analytical chemistry or in chemical analysis of food article. The proper understanding of the terminology involved is very much desired before the start of any analysis.

5.2 What is mean by a Reagent?

A substance which can react with another substance or an agent capable of producing a chemical reaction.

5.3 What is mean by Solution?

A solution is a homogeneous mixture of two or more substances (or components).

The component present in higher proportions is called solvent.

The component present in smaller proportions is called solute.

We generally say that solute is dissolved in solvent to form a solution.

1. A solution in which small amount of solute is present is called dilute solution.
2. A solution in which large amount of solute is present is called concentrated solution.

Fig.5.1 Solution

3. A solution which contains the maximum amount of solute can be dissolved in a given amount of solvent at a particular temperature is called a Saturated solution.

5.4 Standard Solution

A solution whose strength or concentration is known is called as Standard solution.

Concentration or strength of solution can be expressed in different ways:

- Normal solution
- Molar solution
- Molal solution

5.4.1 Normal solution

A solution containing 1gram equivalent (i.e. Eq. wt expressed in gms) of the solute in one litre of the solution is called a normal solution.

5.4.1.1 Normality

The number of gram equivalents of a solute dissolved per litre of the solution. Solute may be acid, base or salt. It is represented by 'N'.

$$N = \frac{gm\ equivalent\ of\ solute}{volume\ of\ solution\ in\ litre}$$

Eq. wt. of solutes i.e. acids, bases & salts is calculated as follows

5.4.1.2 Calculation of eq. wt. of acids

eq. wt. of an acid is generally calculated by dividing the mol wt by its basicity.

Eq. wt of an acid = Mol. Wt/ Basicity
By the basicity of an acid we mean the number of replaceable hydrogen atoms present in one molecule of the acid.

Table 5.1 Property of Solutions

Acid	Mol. Wt	Basicity	Eq.wt
Hydrochloric acid HCl	36.5	1	36.5
Nitric acid HNO_3	63	1	63
Sulphuric Acid H_2SO_4	98	2	49
Oxalic Acid-anhydrous $C_2H_2O_4$	90	2	45
hydrated $C_2H_2O_4.2H_2O$	126	2	63

5.4.1.3 Calculation of eq. wt. of alkalis

The eq. wt of an alkali is often calculated with the help of the expression
Eq. wt of an alkali = Mol. Wt/ Acidity

By the acidity of a base we mean the number of hydroxyl groups present in one molecule of the alkali.

Table 5.2 Properties of Solutions

Alkali	Mol wt	Acidity	Eq. wt.
Sodium Hydroxide NaOH	40	1	40
Potassium Hydroxide KOH	56	1	56
Calcium Hydroxide $Ca(OH)_2$	74	2	37

5.4.1.4 Calculation of eq. wt. of salts like carbonates and bicarbonates

The eq. wt. of salts like carbonate or bicarbonate can be calculated from the equation of its reaction with an acid.

For example, see the equation given below

$Na_2CO_3 + 2HCl = 2NaCl + H_2O + CO_2$

106 2x36.5

This equation shows that two gram equivalents of HCl react with one gram molecule of Na_2CO_3

Eq. wt. of Na_2CO_3 = Mol. Wt/2 = 106/2 = 53

Similarly the eq. wt. of K_2CO_3, $NaHCO_3$ and $KHCO_3$ can be calculated from their equations

Table 5.3

Substance	Mol. Wt.	Total Valency of metal atom	Eq. wt.
Sodium carbonate Na_2CO_3	106	2	53
Potassium carbonate K_2CO_3	138	2	69
Sodium bicarbonate $NaHCO_3$	84	1	84
Potassium bicarbonate $KHCO_3$	100	1	100

In general, the Eq.wt. of salts

$$Eq.Wt\ of\ salt = \frac{Mol.wt.of\ salt}{Total\ valency\ of\ metal\ atom}$$

For example,

Sodium in Na_2CO_3 has total valency of 2
Sodium in $NaHCO_3$ ---------- 1
Potassium in K_2CO_3 ----------2
Potassium in $KHCO_3$ ----------1

5.4.1.5 Mole

The quantity of a substance which contains one gram **formula weight (or molecular weight or formula mass or molecular mass)** of the substance.

5.4.2 Molar solution

A solution which contains one mole (Mol. wt., if the substance is molecule or At. wt., if the substance is atom) of a solute per litre of the solution is called a Molar Solution.

5.4.2.1 Molarity

The number of moles of solute dissolved per litre of the solution. It is denoted by M

M = mole of solute/volume of solution in litre

5.4.3 Molal solution

A solution which contains one mole solute dissolved in a Kilogram of solvent. The total may be more than or less than one litre, depending upon the density of a solvent.

Solvent = 1000gm (1Kg), Solute = 1 mole

Molality = the number of moles of solute dissolved per 1000gm of solvent. It is denoted by 'm'

m=(No.of moles of solute)/(wt.of solvent (K.g)

Note Normality and molarity are temperature dependent, where as molality is temp independent.

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Lesson 6
PREPARATION AND STANDARIZATION OF SOLUTIONS

6.1 Introduction

Standard solutions are the solutions with known strength. The calibration of other solutions and reagents depends upon the accurate strength of these solutions. These solutions are prepared by using certain substances having typical characteristics. These substances are known as standard substances. In this chapter, the properties of these substances and how to prepare standard solution has been discussed.

6.2 Standard Substances

There are two types of substances which are generally employed for preparing standard solutions

- Primary standard substances
- Secondary standard substances.

6.2.1 Primary standard substances

Those substances which can easily be obtained in pure and crystalline form e.g. Oxalic acid, sodium carbonate etc, are called primary standard substances.

6.2.1.1 Characteristics of primary standard substances

- It should be easy to obtain, to purify and to preserve.
- It must not be hygroscopic.
- It should not decompose at ordinary temperature.
- It should be readily soluble under the conditions in which it is used.
- Its reaction with other reagents should be quantitative and practically quick.
- It should have high eq. wt. so that the error due to weighing is minimized.
- It should be fairly cheap.

Fig 6.1 Sodium Carbonate

6.2.1.2 Primary standard substances used for Acid- Alkali titration

a) Sodium carbonate

Since, Sodium carbonate can be easily obtained in pure state, its standard solution is prepared by directly dissolving a known wt of it in water and making the solution a known volume. Eq. wt. of sodium carbonate (anhydrous) being 53, its N/10 solution would contain 5.3 gm/litre and N/20 solution would contain 2.65 gm/litre.

These standard solutions are used for finding the strength of solutions of HCl, H_2SO_4 etc whose standard solutions cannot be prepared directly.

b) Oxalic acid $(COOH)_2$

Oxalic acid is available in pure state and its standard solutions can, therefore, be prepared by the direct method. Eq. wt. of hydrated oxalic acid ($C_2H_2O_4.2H_2O$), being 63 its N/10 solution would contain 6.3 gm/litre, and N/20 solution would contain 3.15 gm/ litre.

These standard solutions are employed to find the strength of solutions of alkalies (NaOH and KOH) whose standard solutions cannot be prepared by the direct method.

6.2.1.3 Preparation of some primary standard solutions

Standard solutions are prepared by using standard substances. Here a known quantity of standard substances depending upon the requirement is dissolved in a known amount of water and desired volume is made. Since, these substances have a constant weight, high purity, non hygroscopic property, so the solution obtained is of known and definite concentration. The examples of such solutions are as follows:

a) Example 1. Standard N/10 oxalic acid solution (Primary standard)

To prepare N/10 solution of oxalic acid, weigh 6.3 gm of oxalic acid & dissolve in distilled water & finally make up the volume to one liter in a volumetric flask. The standard solution of oxalic acid (Primary standard) is used to find the strength of solutions of alkalies like NaOH, KOH (Secondary standards) whose standard solutions can not be made by direct weighing.

b) Example 2. N/10 standard Na_2CO_3 solution (Primary standard)

It can also be obtained in pure form and its anhydrous form is available. Here also, its standard solution can be prepared by direct weighing & dissolving in water to make up to known volume.

To prepare N/10 Na_2CO_3, weight exactly 5.3 gm of pure anhydrous salt, dissolve in distilled water and make up to 1 litre in volumetric flask. Its standard solution is used to find out the strength of solutions of acids like HCl, H_2SO_4, HNO_3 etc whose standard solutions cannot be prepared directly.

6.2.2 Secondary standard substances

Those substances or reagents which cannot be obtained in a sufficient pure state, e.g. NaOH, KOH, HCl, H_2SO_4 are called secondary standard substances.

6.2.2.1 Preparation of some secondary standard solutions

N/10 NaOH: Prepare concentrated stock solution (say 50%) of NaOH by dissolving equal parts of NaOH pellets (50 gm) & water (50 gm) in a flask. Keep it tightly stoppered for 3-4 days. Use the clean, supernatant liquid for preparing N/10 solution. Approximately 8 ml of this stock solution (50%) is required per litre of distilled water. This will give approximate solution. Now take 10 ml of standard N/10 oxalic acid (primary standard) solution in conical flask and add 2- 3 drops of phenolphthalein indicator. Take unknown solution i.e. approximate N/10 NaOH solution in burette and add to the conical flask containing standard oxalic acid solution by continuous mixing

by swirling the flask till the appearance of pink color. NaOH is taken in burette and standard oxalic acid in conical flask as shown below in figure.

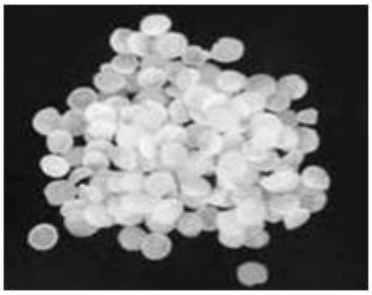

Fig 6.2 Sodium Hydroxide pellets

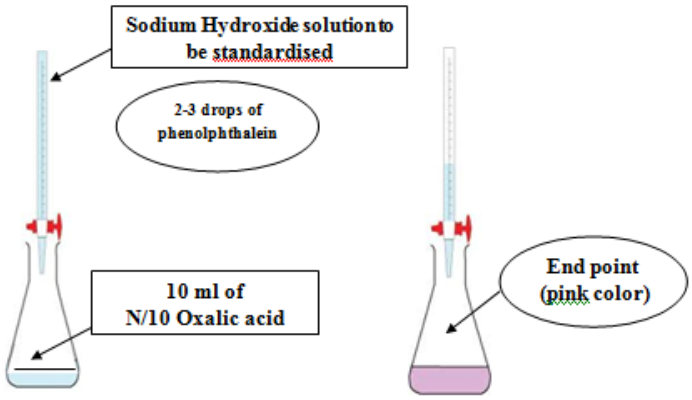

Fig. 6.3 (a) Standard Solutions

Note down the volume of approximate N/10 NaOH solution used in the titration of 10 ml of standard oxalic acid. Calculate the normality of the unknown sodium hydroxide solution by using following equation:

$$N_1V_1 = N_2V_2$$
(Base) = (Acid)

N1 = Normality of NaOH solution. (?)
V1 = Volume of NaOH solution used. (ml)
N2 = Normality of standard oxalic acid solution. (0.1 N)
V2 = Volume of standard oxalic acid solution. (10 ml)

$$N_1 = \frac{N_2V_2}{V_1}$$

If the volume of approximate NaOH used in the titration is less than 10 ml, means the solution is strong and its normality is not N/10, so dilute the basic solution and again standardize with standard oxalic acid solution till normality of approximate solution is same as that of standard solution.

6.3 Approximate Strength of Concentrated Acids Generally Available

Table 6.1 Concentrated acids

Sr. No.	Acids	Sp. Gr.	Approx. Strength
1	Acetic acid glacial CH_3COOH	1.05	16N
2	Hydrochloric acid HCl	1.16	12N
3	Nitric acid HNO_3	1.42	16N
4	Sulphuric acid H_2SO_4	1.84	36N

6.3.1 N/10 HCl

a) Prepare approximately 0.1 N solution on the basis of the strength given on the label by diluting it 120 times with distilled water. Then standardize it against standard N/10 Na_2CO_3 using methyl orange as an indicator. Alternatively, it can also be prepared as given below in "b".

b) Prepare approximately 0.1 N solution on the basis of the strength given on the label by diluting it 120 times with distilled water. Then standardize it against standard N/10 NaOH which is already standardized against N/10 oxalic acid using Phenolphthalein indicator.

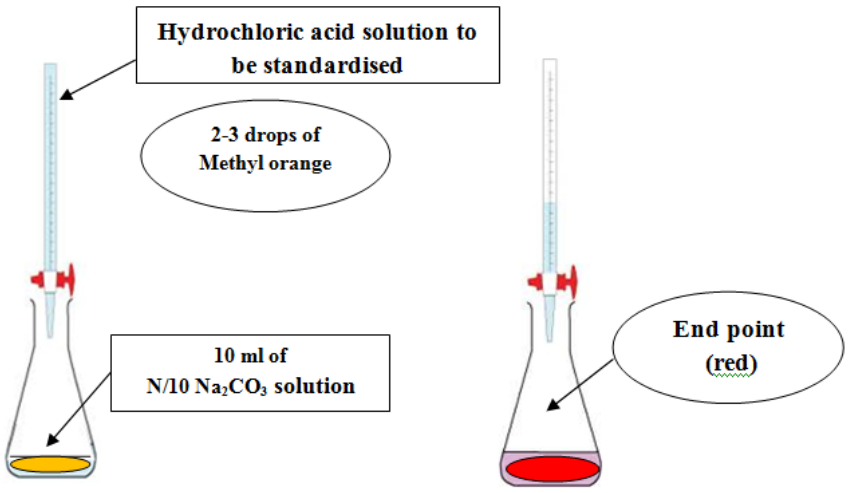

Fig. 6.3 (b) Standard Solutions

6.3.2 N/10 H_2SO_4

Caution:

Concentrated H_2SO_4 is very corrosive in nature, therefore, it should be handled carefully. Always remember: "ADD ACID TO WATER" under cold conditions. This is done to avoid bumping due to the heat generated.

For the preparation of N/10 H_2SO_4, take 10 ml of concentrated H_2SO_4 (usually about 36 N), dilute

36 times by adding acid in small quantity to distilled water in a cold water bath, to make it 1N and then dilute this 1N solution further 10 times to make it N/10. Then standardize against standard N/10 NaOH or N/10 KOH using phenolphthalein indicator.

6.3.3 N/10 HNO_3

Take 10 ml of concentrated HNO_3 (usually about 16 N), dilute 16 times by adding acid to distilled water to make it 1N and then dilute this 1N solution further 10 times to make it N/10. Then standardize against standard N/10 NaOH or N/10 KOH using phenolphthalein indicator.

6.4 Preparation of Some Other Reagents

6.4.1 Preparation of chromic acid (Cleaning solution)

Fig. 6.4 Chromic Acid

Caution:

During preparation of chromic acid, observe the precaution for handling of concentrated H_2SO_4, that is, "ADD ACID TO WATER" under cold conditions.

Dissolve 50 gm of $K_2Cr_2O_7$ in 50 ml of water in a beaker. Keep the beaker in cold water and add slowly 500 ml of concentration H_2SO_4 and cool. It is a very corrosive solution and care should be taken to avoid its coming in contact with the skin. It is almost a saturated solution of $K_2Cr_2O_7$ in concentrated H_2SO_4.

Before using chromic acid, it is necessary to clean the glassware with detergent for the economic use of chromic acid.

6.4.2 Preparation of Gerber sulphuric acid

During preparation of Gerber sulphuric acid, observe the precaution for handling of concentrated H_2SO_4, that is, "ADD ACID TO WATER" under cold conditions.

Gerber sulphuric acid is used to dissolve casein in milk. If dilute H_2SO_4 is used, casein is precipitated but not dissolved, whereas, if concentrated H_2SO_4 is used, it causes charring of organic matter. Therefore, the concentration of sulphuric acid is so adjusted that it is just strong enough to dissolve the casein without charring the fat. The acid also produces necessary heat to keep the fat in the liquid state.

Gerber H_2SO_4 has strength of about 90-91% corresponding to a Sp. Gr. of 1.807 to 1.812, whereas concentrated H_2SO_4 is generally 97-99% (Av 98%) with Sp. Gr. of 1.835.

Therefore, for practical purposes generally 900 ml of concentrated H_2SO_4 is added to 100 ml water to give 1 litre of Gerber acid.

6.4.2.1 Preparation

Take required vol. of water (say 100 ml) in a flask and keep it in a basin of ice-cold water. Carefully, add the required quantity of concentrated H2SO4 (say 900 ml) in small quantities at a time keeping the container sufficiently cold. Mix gently.

6.4.3 Testing the amyl alcohol (a by product of fuel oil refinery) used for fat determination

Iso-amyl alcohol (also called Iso-butyl carbinol) used in Garber fat test shall be clear, colorless and free from impurities particularly fatty mater. Perform various tests to know its purity.

1. Density: At 27°C, Density shall be between 0.803 to 0.805 gm/ml.
2. Boiling point: Boiling point shall be 128-129°C (can be checked using boiling point apparatus).
3. 95% of the liquid shall get distilled between 130-132°C.
4. Test for absence of furfural and other impurities: 5 ml iso amyl alcohol + 5 ml H_2SO_4 (97%) → Observe the color → shall not show more than a yellow or light brown color.
5. Test for absence of fatty matter: Carry out a blank Gerber fat test using distilled water in place of milk. If any fat separation is observed → indicates impurities of some fatty matter.

6.4.4 Preparation of 0.1 N sodium thiopsulphate solution ($Na_2S_2O_3.5H_2O$)

Dissolve approximately 24.8 gm of sodium thiosulphate crystals in previously boiled and cooled distilled water and make the volume to 1000 ml. Store the solution in a cool place in a dark colored bottle. After storing the solution for about two weeks, filter if necessary and standardize as follows:

Weigh accurately about 5.0 gm of finely ground potassium dichromate which has been previously dried to a constant weight at 105 ± 2° in to a clean 1.0 litre volumetric flask. Dissolve in water make up to the mark; shake thoroughly and keep the solution in dark place. Pipette 25.0 ml of this solution into a clean glass stoppered 250 ml conical flask. Add 5.0 ml of concentrated hydrochloric acid and 15.0 ml of 10% potassium iodide solution. Allow to stand in dark for 5 minutes and titrate the mixture with the solution of sodium thiosulphate using starch solution as an indicator towards the end. The end point is taken when blue color changes to green. Calculate the normality (N) of the sodium thiosulphate as follows:

$$N = \frac{25W}{49.03\ V}$$

W = weight in g of the potassium dichromate
V = volume in ml of sodium thiosulphate solution required for the titration.

6.4.5 Preparation of 0.1 N standard solution of silver nitrate

Dissolve slightly more than the required quantity (17.2 g instead of 16.989 g) of reagent grade silver nitrate in distilled water and dilute to one litre in a volumetric flask. Weigh accurately 0.5844 g of NaCl (dried at 110°C before weighing) and transfer to a 100 ml volumetric flask and add 50 ml of halogen free water to dissolve the material. Make up the volume with distilled water to the mark and mix the contents. Pipette out 10 ml of the prepared standard sodium chloride solution in 100 ml conical flask and add 2-3 drops of potassium chromate indicator (5% solution in water). Titrate with silver nitrate solution until a perceptible reddish brown color appears. Carry

out a blank titration using 10 ml of distilled water instead of sodium chloride solution and deduct the blank reading from the reading for the standard sodium chloride solution.

Calculate the normality of the silver nitrate solution using normality equation:

$$N_1V_1 = N_2V_2$$

Where,

N_1 = Normality of standard sodium chloride solution (0.1N)
V_1 = Volume in ml of sodium chloride used for titration (10 ml).
N_2 = Normality of prepared silver nitrate solution.
V_2 = Volume in ml of prepared silver nitrate solution used for titration.

6.4.6 Preparation and standardization of EDTA solutions

1. Preparation of 0.01 M EDTA solution : Dissolve 3.8 g of disodium ethylene diamine dihydrogen tetraacetate (EDTA, M.Wt. 372.25) in distilled water and volume is made to 1 litre. Mix it well, store in polyethylene reagent bottle. It is standardized against 0.01 M $CaCO_3$ or $CaCl_2$.
2. Preparation of 0.01 M $CaCl_2$ solution: Prepare standard Ca solution (1 ml = 1 mg $CaCO_3$, M.wt. 100) by weighing 1 g $CaCO_3$ into 500 ml conical flask or beaker and adding dilute HCl through funnel until $CaCO_3$ is dissolved. Add 20 ml water, boil to expel CO_2 and cool. Add few drops of methyl red indicator and adjust colour intermediate orange (brownish red) with dilute NH_4OH or HCl as required. Transfer quantitatively to 1 L volumetric flask and make up volume to the mark. Shake it well and store it well and store in air-tight reagent bottle.
3. Erichrome Black T indicator: Dissolve 0.5 g of Erichrome black T in 100 ml of triethanolamine. Or 0.4 g in 100 ml methanol.
4. Buffer solution: Dissolve 16.9 g NH_4Cl in 143 ml NH_4OH, and dilute to 250 ml with water. Store in tightly stoppered Pyrex of plastic bottle. Dispense from bulb-operated pipette. Discard after 1 month or when 1-2 ml added to sample fails to produce pH 10.0±0.1 at end point titration.

6.4.7 Standardization of EDTA solution

Rinse and then fill burette with prepared EDTA solution. Pipette 25 ml of standard $CaCO_3$ solution into 250 ml Erlenmeyer flask, add 1 ml ammonia buffer (to raise the pH as reaction takes place at high pH) and 3-4 drops of Erichrome black T indicator. Titrate the EDTA solution until colour changes from wine red to dark blue with no reddish tinge remaining. Calculate the molarity of EDTA ($M_1V_1 = M_2V_2$), if excess follows the procedure for the standardization, recheck the molarity and it should be 0.01 M.

6.4.8 Preparation of Fehling solution

Fehling solution used for the estimation of reducing sugars is generally prepared fresh by mixing equal quantities of Fehling's A and Fehling's B which are prepared separately as follows:

a) Fehling's A

Dissolve 34.639 g $Cu(SO)_4.5H_2O$ in distilled water and add 0.5 ml concentrated H_2SO_4, mix and make the volume to 500 ml. Filter if necessary.

b) Fehling's B

Dissolve 173 g of Rochelle salt (Na K tartarate) and 50 g of NaOH in distilled water. Allow to stand for two days. Filter if necessary.

6.4.8.1 Standardisation of Fehling's solution

1. Pipette 5 ml of Fehling's solution A and 5 ml of Fehling's solution B using two separate pipettes in a 250 ml Erlenmeyer flask. Fill up a burette with the standard lactose solution and connect the burette end with an offset tube to keep the burette tube out of steam.
2. Heat the content of the flask to boiling over burner or heater and maintain moderate boiling for 2 min. To prevent bumping add some inert boiling chips. Add 3 to 4 drops of methylene blue indicator (0.2% in water) without removing from the flame. Titrate the content of the flask against standard lactose solution (0.5%) from the burette until the blue colour disappears and the bright brick-red colour of precipitated Cu_2O appears (at the end point the Cu_2O suddenly settles down giving a clear supernatant). Note the volume of lactose solution required for the standardization of Fehling's solution. After this preliminary titration, further titration or titrations should be carried out, adding practically the whole of the standard lactose solution volume (one ml less than required as observed in first titration) required for the titration before commencing the heating. Let the contents boil for 2 minutes. Now, add 3-4 drops of methylene blue indicator, continue heating and complete the titration within 3 min from the commencement of boiling. Let V_1 ml be the titre for this experiment.

Note: Carefully note the first disappearance of blue colour. Once missed, it is difficult to ascertain the end point. Maintain the boiling at a uniform rate during the titration.

Multiply the titre value by mg/ml lactose of the standard solution to obtain total lactose required to reduce the copper and term the value a "Factor F".

6.4.9 Preparation of pH indicator solutions:

6.4.9.1. Phenolphthalein indicator solution: - Weigh 1.0 gm phenolphthalein and place the powder in a 100 ml volumetric flask containing about 50ml of 95% ethanol. Stopper and shake vigorously for a few minutes, then add 20ml more ethanol and shake until a clear solution is formed and make the volume to 100 ml.

6.4.9.2. Methyl orange indicator solution: Dissolve 1.0 gm of methyl orange powder in distilled water and dilute to one litre. Filter, if necessary.

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Lesson 7
SAMPLING PROCEDURES; LABELING OF SAMPLES FOR ANALYSIS AND CHOICE OF ANALYTICAL TESTS

7.1 Introduction

Sampling of milk and milk products shall be done by an experienced person who is familiar with the techniques and is well acquainted with the knowledge of the subject. It is not possible to lay down a single sampling procedure which will be applicable in all the cases. The sampling procedure, therefore, differs according to the nature of the material and the purpose for which it is needed. Sampling may be required for chemical or bacteriological examination. All precautions shall be taken to prevent contamination and adulteration. For chemical examination, the sampling equipment shall be clean and dry. For bacteriological examination, all equipments including plunger, sample bottles and rubber stoppers shall be sterile and the samples shall be collected under aseptic conditions.

If subsequent analysis or interpretation is to be of some value, it is very important that sample should be a true representative of the bulk. Since milk fat is of lower density than the other constituents of milk, it tends to rise to the surface. Therefore, thorough mixing of milk with a proper instrument which will reach the entire depth of the liquid is essential to ensure a representative sample of the entire batch. In small batches, it should be possible to accomplish mixing by pouring the entire quantity of milk from one container to another, three or four times. Larger batches of milk shall be thoroughly agitated by a hand stirrer or by mechanical means. Milk churns easily at 26.5 to 29.5°C and agitation near this temperature shall be avoided.

7.2 Sampling from Individual Container

Pour the milk from one container to another, three or four times. Where this is not practicable, mix thoroughly with a plunger. In mixing the milk, the plunger shall be allowed to fall to the bottom of the container and brought to the top of the milk as rapidly as possible not less than 10 times. The position of the plunger shall also be moved from place to place to ensure that the whole of the milk at the bottom of the vessel is thoroughly agitated and mixed with the upper layer. Any milk fat adhering to the neck and under the shoulder of the can shall be well mixed with the remainder of the milk. After thorough mixing, a sample shall be drawn immediately.

Fig. 7.1 Sampling from individual container

7.2.1 Sampling from several containers

The sample shall be taken after pouring the contents of the containers into a vat and mixing. When this is not possible, a composite sample is taken in the following manner from the containers after milk has been agitated and mixed. First, the milk shall be distributed as equally as possible among a number of containers. The cans shall not be filled, but the same quantity shall be placed in each.

After mixing the contents of each can thoroughly, an equal volume of milk shall be taken from each. These portions shall be placed in another vessel, thoroughly mixed as described in case of individual container and a sample then taken.

Alternatively, where facilities exist for accurate measurements, a composite sample may be obtained by taking the same proportion of the milk therein from each container in a consignment after thorough mixing, collecting this in another vessel and taking a sample as described in case of individual container.

Fig. 7.2 Sampling from several containers

7.2.2 Sampling Bulk Units

When milk of uniform quality is supplied in bulk units (for example, cans filled from storage tanks), the number of random units to be sampled shall be as follows:

Table 7.1 Unit selection in testing laboratory

Total Number of Units	Number of Units to be Selected
1	1
2-5	2
6-20	3
21-60	4
61-100	5
Over 100	5 plus one for each additional 100 units or fraction thereof

The testing laboratory, may, within its discretion, instruct the person who draws the sample to submit separate samples from each unit selected, or one or more composite samples consisting of aliquot portions from each unit selected.

The latter course should only be applied where the product is likely to be of fairly uniform composition, for example, where the consignment to be sampled is produced from a quantity of properly mixed milk, and where variations in composition from unit to unit are, therefore, small. But, where there is a possibility of wide variations between different units, every selected unit shall be separately sampled.

7.2.3 Sampling from storage tanks and rail and road milk tankers

The method of sampling of milk from storage tanks and rail and road tankers is largely governed by storage/transport conditions. It is, therefore, difficult to lay down any rigid procedure for the sampling, but the following is recommended:

Fig. 7.3 Sampling from storage tanks and rail tankers

In all cases, the milk in the tank/tanker shall be thoroughly mixed by a sufficiently large plunger, a mechanical agitator or by compressed air; the uniformity of the samples being determined, when necessary, by mixing till such time as complete agreement is obtained between samples taken at the manhole and at the outlet cock in respect of fat and total milk solids.

NOTE- When a plunger is used for mixing the milk in rail or road milk tankers, a convenient and satisfactory method is to insert the plunger in the man-hole, the operator sitting or standing astride (with the legs apart on each side) on top of the tanker. The plunger is thrust forward and pulled back, thrust downwards and pulled back and thrust backwards and pulled back. The cycle of operations should be repeated for at least 15 minutes.

Fig. 7.4 Plunger

After proper mixing of the milk, the sample may be taken from the tank, removed through the stopcock in the tank door, or from a valve on the discharge line from the tank when it is being emptied.

7.2.4 Composite Milk Samples for Fat test

Suppliers of milk are often paid for milk on the basis of fat test. The determination of fat contents of the suppliers' daily deliveries is laborious and expensive. Therefore, composite samples of the suppliers' milk are taken over a period and then tested. After thorough mixing, proportionate amounts of the suppliers' daily delivery are collected and placed into the patron's composite sample bottle. The total volume of the individual composite sample shall be not less than 175 ml.

For preserving the composite sample, 0.1 ml of 36 percent formaldehyde for 25ml of milk may be used. The bottle containing the composite milk sample shall be tightly stoppered to prevent evaporation and kept in a locker, away from light, till required for analysis. The sample shall be analyzed on the same day as the last portion of milk is transferred to the composite sample bottle.

NOTE – Each time when fresh sample of milk is added, the sample shall be mixed by rotating the bottle to prevent the formation of solid cream layer or cream plug.

7.3 Treatment of Milk Sample on Arrival at the Laboratory before Analysis

Warm the sample in the bottle to about 40°C in a water bath and mix thoroughly. Cool to 26° - 28°C. Leave aside the sample for about 4 minutes after mixing to allow air bubbles to rise and escape. After that, mix the sample by inverting the bottle 3-4 times and start analysis.

7.3.1 Preparation of cream sample for analysis

When cream is thin and in small containers, it shall be mixed either by six transfers, or by plunging not less than ten times. The position of the plunger shall be moved from place to place to ensure that the whole of the cream at the bottom of the vessel has been thoroughly agitated and mixed with the upper layer. To avoid whipping and churning, the disc of the plunger shall not be brought above the surface of the cream.

When cream is thick or in bulk containers, it shall be mixed by plunging as described for thin cream.
When the cream is sour, the material shall be warmed so as to attain a temperature between 30° and 40°C and, while cooling it to room temperature, the container shaken gently or the contents stirred. Keep the contents covered as much as possible.

In all cases the sample shall be taken immediately after mixing.

7.4 Sampling of Paneer/Cheese/Chhana
One of the following three methods is employed

7.4.1 Sampling by cutting a sector
Using a knife with a sharp blade, two random cuts are made radially proceeding from the centre of the cheese/paneer towards the edge.

7.4.2 Sampling by means of a trier
The cheese trier is driven obliquely into the surface of the paneer or cheese towards the centre

once or several times at a point at least 10 to 20 cm from the edge of the cheese. From the boring or borings thus obtained a part of at least 2 cm length is cut off together with the crust and is used to close the hole of the cheese. The remaining portions of the boring or borings constitute the sample.

Fig. 7.5 Trier

However, when the cheese is delivered in drums, cases or other larger containers, sampling may be carried out by driving the trier obliquely through the content of the container from the top to bottom. This method is suited for sampling of processed cheese.

7.4.3 Sampling by taking a whole cheese

This method is made use of for cheese packed in small containers.

7.5 Preparation of Paneer/Cheese/Chhana Sample for Analysis

Samples shall be prepared for chemical analysis by passing them quickly through a suitable grater, by grinding them quickly in a mortar and returning them to the sample container or by cutting them into small pieces with a sharp knife in the container.

7.5.1 Cheese

7.5.1.1 Sampling by cutting

Use knife with a pointed blade

7.5.1.2 Circular base

Make two cuts radiating from the centre of the cheese, when cheese is circular in shape, after removing the inedible portion sample should be minimum 150 gm.

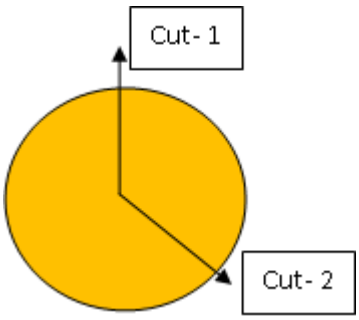

Fig. 7.6 Cutting of cheese

7.5.1.3 Rectangular base

Make cuts parallel to the sides. After removing the inedible portion sample should be minimum 150 gm.

Fig. 7.7 Cutting of cheese on rectangular base

7.5.1.4 Sampling by using trier

Use trier

i) Insert Trier obliquely towards the centre of the cheese into one of the surfaces at a point not less than 10 cm from the edge.

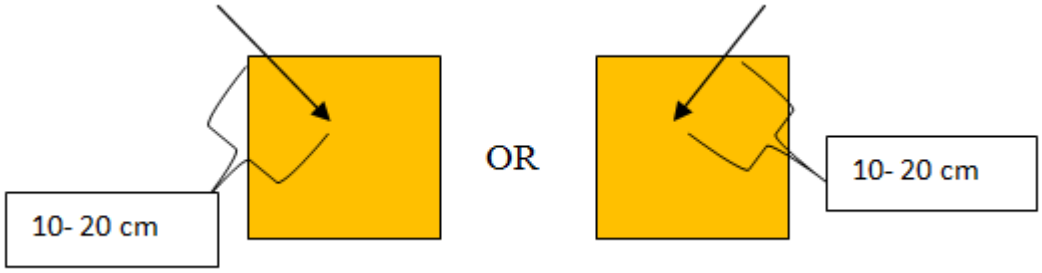

Fig. 7.8 Sampling by insertion of Trier towards center of cheese

ii) Insert Trier horizontally into the vertical face midway between the two plain faces, towards the centre of the cheese

iii) Insert Trier perpendicularly into one face and pass through the centre of the cheese to reach the opposite face.

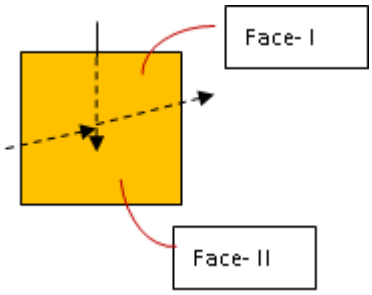

Fig. 7.9 Sampling by insertion of Trier perpendicularly

All samples should be prepared for chemical analysis by passing them quickly through a suitable grater, by grinding them quickly in a mortar and returning them to the sample container or by cutting them into small pieces with a sharp knife in the container.

7.6 Sampling of Khoa

The sampling of khoa follows the procedure used for sampling cheese/paneer/chhana, except that a clean dry stainless steel knife with sharp pointed blade is used to cut khoa for sampling.

7.7 Sampling of Condensed Milk

7.7.1 Scale of sampling

The number of containers to be selected from each lot shall be as follows:-

a) For containers of 400 gm to 5 Kg.

Lot Size (N) No. of containers to be selected

Upto 300 3

301 – 500 5
501 – 1000 7
1001 and above 10

b) For containers of more than 5 Kg and upto 20 Kg

Lot Size (N) No. of containers to be selected

Upto 100 2
101 – 300 3
301 – 500 4
501 and above 5

c) The scale of sampling for containers of 200 g and above 20 kg shall be an agreed to between the purchaser and the vendor.

The containers from the lot shall be chosen at random. For example, starting from any container, count them as 1, 2, 3 ------ etc. up to r in one order, where r = N/n (N being the size of the lot and n being the number of containers to be selected). Every r^{th} container thus counted shall be separated until the requisite number of containers is obtained from the lot to give the sample for test.

7.7.2 Preparation of sample of condensed milk for analysis

On storage of condensed milk, separation of the constituents such as fat, lactose may occur. It is necessary to mix the contents of the container prior to analyses in the following manner:

Heat the container in a water bath at about 40°C until the sample has nearly reached this temperature. Open the container at the edge of the lid. Re-incorporate all the material adhering to the lid into the container. Mix the contents thoroughly by stirring with a spoon or spatula, in such a way that the top layers as well as contents of the lower corners are moved and mixed. Repeat the stirring before drawing the sample for testing various parameters.

7.8 Sampling of Milk Powder

7.8.1 Scale of sampling

The no. of containers to be selected from each lot shall be as follows:

a) For containers of 500 gm and upto 5 kg

Table 7.2 For containers of 500 gm and upto 5 kg

Lot Size (N)	Sample Size (For tests other than Microbiology) (n)	Sub sample size(For Microbiology test) (n)
Upto 100	3	1
101 to 300	5	2
301 to 500	7	3
501 & above	9	4

b) For containers of more than 5 kg

Table 7.3 For containers of more than 5 kg

Lot Size (N)	Sample Size (For tests other than Microbiology) (n)	Sub sample size(For Microbiology test) (n)
Upto 100	2	1
101 to 300	3	1
301 to 500	4	2
501 & above	5	3

7.8.2 Preparation of sample of milk powder for analysis

Draw with a suitable sampling instrument approximately equal quantity of the material from different parts of the same container till about 150 g of the material is obtained. Transfer the material immediately to thoroughly clean and dry container and seal air-tight.

7.9 Sampling of Ice-Cream

7.9.1 Scale of sampling

The no. of containers to be selected from each lot shall be as follows:

A) When the product is supplied in bulk units

Table 7.4 Product is supplied in bulk units

Total Number of Units (N)	No. of Units to be selected (n)
1	1
2 to 5	2
6 to 20	3
21 to 60	4
61 to 100	5
Over 100	5 plus one for each additional 100 units or fraction thereof.

NOTE - When there is a possibility of wide variations between different units, every unit shall be sampled.

B) When the product is supplied in retail units

Table 7.5 Product is supplied in retail units

Total Number of Units (N)	No. of Units to be selected (n)
1 to 100	1
101 to 1000	2
1001 to 10,000	4
Over 10,000	4 plus one for each additional 2500 units or fraction thereof

NOTE - Each batch is to be dealt separately in a similar manner.

7.9.2 Preparation of sample of Ice-Cream for analysis

The samples shall be stored at a temperature not higher than -15°C. During transit the samples shall be maintained at a temperature not exceeding -15°C.
Any sample of ice-cream shall not be less than 100 g. If necessary several packages of smaller size shall be taken to make up the required size of sample.

In the case of multilayered ice-cream, the sample shall be such as to contain the same proportion of each layer as is present in the original ice-cream. Different layers shall not be separated at the time of sampling and a complete sample of all layers shall be placed in the sample jar.

For the purpose of melting, the frozen sample may be kept at room temperature or, if required, in water bath at a temperature not exceeding 45°C for not more than 15 minutes. Thoroughly mix the samples before removal of the test portion.

7.10 Sampling of Butter

Scale of Sampling: The no.of containers to be selected from each lot shall be as follows

A) When the product is supplied in bulk units (like casks or boxes)

Table 7.6 Product is supplied in bulk units

Total Number of Units (N)	No. of Units to be selected (n)
1	1
2 to 9	2
10 to 49	3
50 to 99	4
100 to 199	5
Over 200	5 plus 1 for each additional 250 units or fraction thereo

B) When the product is supplied in small units (like packets or tins)

Table 7.7 Product is supplied in small units

Total Number of Units (N)	No. of Units to be selected (n)
1 to 100	1
101 to 1000	2
1001 to 10,000	4
Over 10,000	4 plus 1 for each additional 2500 or fraction thereof

7.10.1 Sampling technique for chemical analysis

Hard and semi-hard butter kept under cold storage

a) From churns

Four cores shall be drawn with the help of a trier at equal distances. At least two should be near the centre of the churn.

b) From trollies

Four cores (one each from the two ends and the other two from the sides) shall be drawn with the help of a trier.

c) From boxes

Three cores shall be drawn by inserting a trier vertically through the block. One core would be at the centre and the other two near diagonally opposite corners of the open end.

d) From casks

Three cores shall be drawn by inserting a trier at three points equidistant from the circumference of one end of the block and directed through the centre of the block.

e) From small packets

The samples shall consist of the unopened packets. After taking the sample for bacteriological test, the rest shall be used for chemical analysis.

f) Barrel

Butter barrel (a) Insert butter Trier diagonally from the edge of the barrel and rotate the Trier through complete turn and take out Trier with a plug of butter (b) Take another plug by inserting Trier arbitrarily at any point of the surface vertically down to the bottom rotate the Trier through complete turn and take out trier with a plug of butter. Plug the holes with about 25 mm of the plug and use remaining 75 mm of the plug as sample and mix the samples drawn (200 gm) for analysis

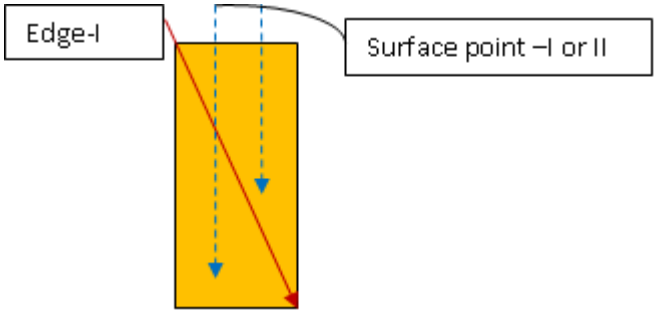

Fig. 7.10 Sampling of butter barrel

i) Block

Insert trier from top corner-I diagonally through out the centre to the bottom and rotate trier through one complete turn and withdraw the full core. Repeat the sequence from top corner-II and plug the holes with 25 mm portion of the butter core drawn.

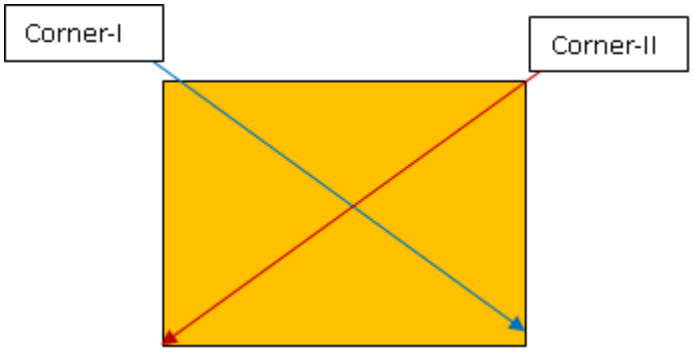

Fig 7.11 Sampling of butter block

ii) Butter in pats or rolls less than 500 gm: In this case take whole unit as sample

iii) Butter in pats more than 500 gm: Divide unit into four parts and take two opposite quarters as sample.

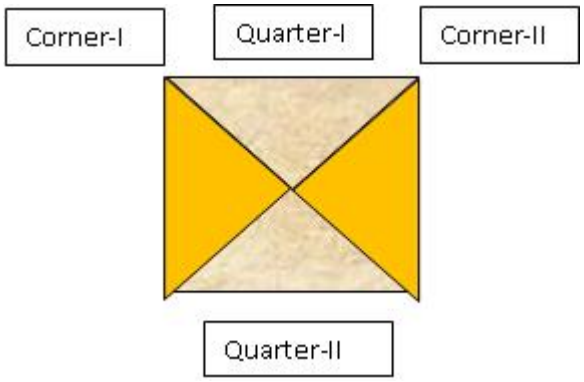

Fig 7.12 Division of butter block in quarters

7.10.2 Pasty butter kept under warm conditions

When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point, located 2 to 3 cm away from the ends. A suitable, clean, dry spoon, spatula or a trier should be used.

When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm away from the bottom and the third at an equal distance from the centre on the opposite side. At each point, draw from the deep layers three cores, roughly equidistant on the circumference. A suitable, clean, dry spoon, spatula or a trier should be used.

7.10.3 Preparation of sample of butter for chemical analysis

7.10.3.1 Sample for analysis of butter

Warm the sample in an air-tight container with the lid screwed down tightly or with the glass stopper, in an oven or water-bath not exceeding 39°C until by frequent vigorous shakings a homogenous fluid emulsion (free from un-softened pieces) is obtained at the lowest possible temperature.

7.10.3.2 Sample for analysis of butterfat

Heat a portion of emulsified butter in a beaker to a temperature of 50-60°C until the fat separates. Filter the fat layer through a dried filter paper into a dry vessel at a temperature above the solidification point of the fat, using a hot-water funnel, if necessary. Re-filter the filtrate under the same conditions, until it is clear and free from water. Liquefy the fat completely and mix before taking samples for analysis.

Note: Exposure to light and air of the butter sample or the butterfat obtained from it shall be as short as possible and analysis shall be carried out without delay.

7.11 Labeling of Samples for Analysis

Each sample container (bottles or jars or other container) shall be sealed air-tight after filling and a label marked with the following particulars should be put on the container:

7.11.1 Purpose of sampling

Sampling of milk and milk products is generally done for Chemical analysis, Bacteriological analysis, Sensory analysis etc. The sample should bear the following information to ensure the tractability of the sample.

Name of the supplier/manufacturer:

Date and time of sampling and place of sampling:

Nature of the product like: Milk sample, Butter sample, Ghee sample, Milk powder etc.

Identification number, name, designation and signature of the person responsible for taking the sample:

Mass or volume of the sample:

Particular of the stock/ unit from which the sample is taken i.e.

Stock number
Batch number
Code number

Preservative added or not to keep the sample suitable/ fit for analysis.

- If yes, then
- The nature of preservative added
- Quantity of preservative added

If no preservative is added, then storage, temperature during transit or transportation till analysis is done, should be mentioned.

e.g.

- Store at refrigerated temperature
- Store at room temperature etc.

Additional Information: if sample is taken from a food which has some certification mark like ISI, Agmark etc, then in that case, give additional information such as

Mark (ISI/ Agmark)

Grade (Special, General etc)

Agmark label no./ Batch no.

Name packing station where the food was packed etc.

7.12 Sampling of Ghee

Sampling shall be carried out by an experienced person. A sample which is representative of the bulk is essential. All the containers in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the group of containers in each batch shall constitute separate lots. The number of containers to be selected for sampling shall depend upon the lot size and shall be in accordance with following Table.

Table 7.8 Sampling ghee containers

Number of containers in lot	Number of containers to be selected
1	1
2 to 40	2
41- 110	3
111-300	5
301-600	7
601 and above	10

These containers shall be selected at random from the lot. Samples drawn from the consignment should be placed in appropriate containers, which could be ***wide mouth jar*** and *bottles* and t***in containers*** of 50, 100 and 200/250 ml capacities. The jars shall be closed by means of a screw cap lined with butter paper. Bottles shall be glass-stoppered. Tin containers shall be closed with the,

press-on type of lids. For chemical analysis, bottles may also be closed with rubber stoppers lined with butter paper if organoleptic tests are not to be made.

For the preparation of composite sample, collect equal quantity from each of the selected containers so as the total quantity is at least 300 gm.

7.13 Choice of Analytical Test

7.13.1 Introduction

Generally an analytical chemist or scientist will confront with the problem of selection of proper method from array of methods for quantitative analysis. A variety of methods may be capable of achieving the desired analysis and the decision to select one may depend on a variety of issues.

There are several factors or criteria or issues which determine the choice of selecting a method from the available number of methods. These are as follows:

- *Speed*

Time taken by a method to complete the analysis is one of the criteria. It should be less time consuming method.

- *Convenience*

The method should be convenient to use. It should not be cumbersome i.e. difficult.

- *Accuracy/Precision*

The method of analysis should give accurate results. That means the method should be error free.

- *Sensitivity/Detection limits of the method*

The method should be sensitive enough to estimate even the small traces of component. Higher the sensitivity better will be the results. Therefore, as far as possible the detection limit should be low means the method should be able to detect lower levels of the components in a food.

- *Selectivity/Non-interference of other compounds present in the sample*

The method should selectively estimate the component which we want to estimate. Other components present in the sample should not interfere in the estimation of a particular component in the sample.

- *Availability of instruments/Specific apparatus*

Instrument/ Specific apparatus required in a selected method should be available in the laboratory or a department where work is being carried out.

- *Amount of sample*

The selected method should be such that only small amounts of sample should be required for analysis.

- *Level of analysis/Nature of analysis*

The method of selection of a particular test will also depend upon the type or nature or level of analysis. For example, the test may be required for qualitative purposes. Generally, separate tests or methods are available for qualitative and quantitative analysis.

In case of quantitative analysis, the selection of a test or method will also depend on whether you want to estimate the given component from a gross composition point of view or a contamination point of view. It may also be required to check the residual level of the component after the processing of a product so as to check the permissible limits of the residual components (e.g. pesticides, antibiotics etc) as per the food laws. For all these purposes different types of methods are required. For example, for the estimation of traces or residual contents, more sensitive methods are required.

1. *Cost of the method*

The method of analysis should be such that the cost of estimation of a component should not be high.

2. *Hazards free/Risk free*

The method selected should be such that there should be no hazard/risk involved in the analysis. There should be appropriate precautions needed to minimize the risk involved, if any.

3. The published literature should be available to choose or select a method.

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Lesson 8
DETECTION OF NEUTRALIZERS, PRESERVATIVES AND ADULTERANTS IN MILK AND MILK PRODUCTS

8.1 Introduction
8.1.1 Adulteration of food products

As per the definition of the Prevention of Food Adulteration Act, 1954 (PFA) Rule 2, "adulterant" means any material, which is or could be employed for the purposes of adulteration.

Any food article is deemed to be "adulterated"

- if the article sold by a vendor is not of the nature, substance or quality demanded by the purchaser and is to his prejudice, or is not of the nature, substance or quality which it purports or is represented to be;
- if the article contains any other substance which affects, or if the article is so processed as to affect, injuriously the nature, substance or quality thereof;
- if any inferior or cheaper substance has been substituted wholly or in part for the article so as to affect injuriously the nature, substance or quality thereof;
- if any constituent of the article has been wholly or in part abstracted so as to affect injuriously the nature, substance or quality thereof;
- if the article has been prepared, packed or kept under insanitary conditions whereby it has become contaminated or injurious to health;
- if the article consists wholly or in part of any filthy, putrid, rotten, decomposed or diseased animal or vegetable substance or is insect-infested or is otherwise unfit for human consumption;
- if the article is obtained from a diseased animal;
- if the article contains any poisonous or other ingredient which renders it injurious to health;
- if the container of the article is composed, whether wholly or in part, of any poisonous or deleterious substance which renders its contents injurious to health;
- if any coloring matter other than that prescribed in respect thereof is present in the article, or if the amounts of the prescribed coloring matter which is present in the article are not within the prescribed limits of variability;
- if the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits;
- if the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities not within the prescribed limits of variability, but which renders it injurious to health;
- if the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities not within the prescribed limits of variability but which does not render it injurious to health provided that, where the quality or purity of the article, being primary food, has fallen below the prescribed standards or its constituents are present in quantities not within the prescribed limits of variability, in either case, solely due to natural causes and beyond the control of human agency, then, such article shall not be deemed to be adulterated within the meaning of this sub- clause.

Further under rule 44, PFA states that no person shall either by himself or by any agent sell milk which contains any added water and also milk containing a substance not found in milk.

Amongst the food items, milk is a complex mixture of nutrients and a liquid food which, can be easily adulterated by the unscrupulous persons. According to PFA definition, "Milk is the normal mammary secretion derived from complete milking of healthy milch animal without either addition thereto or extraction therefore. It shall be free from colostrum. Milk of different classes and different designations shall confirm to the standards laid down for them.

8.2 Adulteration of Milk

8.2.1 Common adulterants

The most of the adulterants added to milk can be grouped in the following categories:

a) Carbohydrates

Sugar, glucose, starch, malto-dextrin, etc.

b) Salts and fertilizers

Urea, ammonium sulphate, ammoniacal fertilizers, potassium sulphate, sodium chloride etc.

Fig. 8.1 Sugar

c) Neutralizers

Sodium carbonate, sodium bicarbonate, sodium hydroxide, calcium hydroxide, etc.

d) Preservatives

Hydrogen peroxide, formalin etc.

e) Detergents

Liquid detergents, washing powders etc.

f) Oils and Paints

Vegetable fats and oils, mineral oil / cutting oil, white paint etc.

Most of these adulterants and preservatives can be detected by the following tests

8.2.2 Carbohydrates

8.2.2.1 Sugar

Take 1ml of milk sample and add 1 ml of 0.5% resorcinol solution (0.5% resorcinol solution in 3N Hydrochloric acid). Mix the contents and heat in boiling water bath for 5 min. If sugar is present in the sample, the red color will be produced. Blank shows pale yellow or brownish color.

8.2.2.2 Starch

Starch being cheaper, is some times added in the milk by adulterators to raise the S.N.F of milk. However, it can be easily detected by using iodine solution. Take 3.0 ml of suspected milk sample in a test tube. Add one drop of 1% iodine solution*(1gm of iodine crystals and 5gm Potassium iodide in 100 ml distilled water, mixed by warming to dissolve the iodine crystals)*. Development of blue color indicates the presence of starch.

Fig. 8.2 Starch

8.2.2.3 Glucose

Reagents

a) Barfoed's reagent *(Prepared by dissolving 24 gm of cupric acetate in 450 ml boiling distilled water and immediately adding 25 ml of 8.5% lactic acid to the hot solution and cooling and diluting the contents to 500 ml).*

Fig. 8.3 Glucose

b) Phosphomolybdic acid reagent. *(Dissolve 35gm of Ammonium molybdate and 5 gm of sodium tungstate in 400ml of 5% (W/V) Sodium hydroxide solution. Boil the contents vigorously for 20 – 40 min so that the reactants are dissolved properly. While boiling, the ammonia is released. To check the ammonia escaping in the vapours, put red litmus paper in the path of vapours, if it turns blue means reagent is still not free from ammonia. No change in the color of litmus paper indicates that reagent is free from ammonia. As a result of boiling, water gets evaporated, now cool the contents and dilute to about 350 ml and add 125ml of concentrated (85%) phosphoric acid. Finally make the volume to 500ml with distilled water).*

Procedure

Glucose is also easily available in commercial form as concentrated syrup, so adulteration of milk with this concentrated syrup is very much prevalent in the industry. To detect glucose in milk, take 1ml of adulterated milk sample in a test tube and add 1 ml of Barfoed's reagent. Heat the mixture for 3 min in a boiling water bath and cool for 3min under tap water. Add 1ml of phosphomolybdic acid reagent and mix the contents. Formation of blue color indicates the presence of glucose.

8.2.2.4 Dextrin/ Maltodextrin

Take 1ml of suspected milk sample in a test tube. Add 1 ml of iodine solution (0.005N Iodine solution prepared using 3% Potassium iodide solution in water). Mix the contents. Development of chocolate red brown color indicates the presence of dextrin/ maltodextrin.

8.2.3 Neutralizers & preservatives

I. Neutralizer

In milk, sodium hydroxide, sodium carbonate and sodium bicarbonate are added by adulterators to neutralize the developed acidity in milk. Their detection can be carried out by employing the following tests:

A) Rosalic acid test

Take 5 ml milk in a test tube of capacity 20 ml. Add 5 ml ethanol (95%,v/v) and 4 drops of Rosalic acid solution (0.05%; w/v in ethanol). Mix the contents. If carbonate or bicarbonate is present a pink color will appear. If NaOH is present a deep rose red color will appear.

Fig. 8.4 Neutralizers

B) Alkalinity of ash test

Take 20 ml milk in a silica crucible. First evaporate the water to dryness, and then burn the content to ash in a muffle furnace at 550°C. Disperse the ash in 10 ml distilled water and titrate the ash content against N/10 HCl using phenolphthalein as indicator. If the volume of N/10 HCl exceeds 1.20 ml, then the milk contains the added neutralizers.

II. Formaldehyde

Formalin (40% water solution of formaldehyde) is generally used by Public Health Departments to preserve the milk samples for chemical analysis purpose. Formaldehyde is very poisonous chemical. Though, it can preserve the milk for very long time, it should never be added to milk mean for human consumption due to its poisonous property. Moreover, it affects the quality of the milk products. If milk kept at room temperature (25 to 35°C) for longer time, did not sour, then that milk must be suspected for the presence of formaldehyde. Formaldehyde added to milk can be tested by the following simple test.

Hehner Test: Take 5 ml of the suspected milk sample in a test tube. Add gently 2 ml of sulfuric acid containing trace of Ferric chloride along the side of the tube (the added acid should form a bottom layer of the mixture without mixing the milk). Formation of a violet to purple colored ring at the junction of the two liquids indicates the presence of formalin in the milk sample.

Chromotropic acid test: Take one ml of milk in a test tube and add one ml of chromotropic acid reagent *(reagent is prepared by dissolving 500 mg of chromotropic acid (1, 8-dihydroxynapthalene-3, 6 disulphonic acid)*, in 72 per cent sulphuric acid). Mix the contents well. Presence of formalin in milk is cofirmed by the appearance of yellow color; whereas, control sample remains colorless.

Leech test: Take about 5.0 ml of milk in a test tube. Add to it equal volumes of concentrated HCl containing 1.0 ml of 10% ferric chloride solution to each 500 ml of the acid. Heat over a flame for about five minutes. Rotate the tube to breakup the curd, and observe the color. Development of violet color indicates the formaldehyde added as a preservative.

III. Hydrogen peroxide

Take 5 ml milk in a test tube and then add 5 drops of 2% paraphenylene diamine solution and shake it well. Change of the color of milk to blue confirms that the milk is added with hydrogen peroxide.

8.2.4 Fertilizers and salts

A) Urea

i) Take 5 ml of milk in a test tube and add 5 ml of 1.6% DMAB reagent (prepared by taking 1.6 g of p-dimethyl aminobenzaldehyde in 100 ml of ethyl alcohol containing 10 ml of concentrated hydrochloric acid). Appearance of distinct yellow color indicates the presence of added urea whereas development of slightly yellow color is due to natural urea in milk.

Fig. 8.5 Urea test

ii) Take 5 ml of milk in a test tube and add 0.2 ml of urease (20 mg/ml). Shake well at room temperature and then add 0.1 ml of bromothymol blue solution (0.5%). Appearance of blue color after 10-15 min indicates the adulteration milk with urea.

B) Pond water

This method actually detects nitrates present in the pond water. In the pond water nitrates may come from fertilizers used in the fields.
Rinse a test tube with the suspected milk sample. Along the side of the test tube add about 1 or 2 drops of 2% solution of diphenylamine. The sides of the test tube will turn blue if the milk sample contains pond water.

Fig. 8.6 Pond water

C) Ammonium compounds

Ammonium compounds are detected by using Nessler's reagent.

Nessler's reagent

Dissolve 50gm of potassium iodide in the smallest possible amount of distilled water, add a saturated solution of mercuric chloride until an excess is indicated by the formation of reddish precipitate. Add 900 ml of 50% solution of potassium hydroxide. Make up the volume to one litre. Allow the contents to settle down and take the clear supernatant.

Take 1ml milk adulterated with ammonium compounds. Add 1 ml of Nessler's reagent. Mix the contents and observe the color after 1.5 min and before 2 min. Development of characteristic red brown color is an indication of the presence of ammonium compounds in milk.

D) Common salt

Detection of common salt is based on the principle of argentometric titrations. In this case excess of silver nitrate is made to react with potassium chromate and resulted in the formation of reddish brown precipitates of silver chromate.

Take 5ml of milk in a test tube. Add 1 ml of silver nitrate solution (4.3 gm/100ml double distilled water). Mix it and add few drops of 5% potassium chromate indicator in water and mix the contents. Observe the color change. Development of yellow color indicates the presence of excess salt and red color indicates the absence of excess sodium chloride.

8.3 Detergents in Milk

i) Take 2.0 ml of milk in a test tube and add 1.0 ml of 0.025% methylene blue solution in water. Mix the contents and add 5.0 ml of chloroform and shake the contents vigorously. Keep the tubes undisturbed for 15 min or centrifuge the contents for 10 min. and observe the color in lower layer. Intense blue color in lower layer indicates the presence of detergent.

Fig. 8.7 Detergent

8.4 Test for Detection of Ammonium Sulphate

Take 1.0 ml of milk; add 0.5 ml of 2% sodium hydroxide, 0.5 ml of 2% sodium hypochlorite and 0.5 ml of 5% phenol solution. Heat for 20 seconds in boiling water bath, bluish color turns deep blue in presence of ammonium sulphate. The development of pink color shows that the sample is free from Ammonium sulphate.

8.5 Adulteration of Milk Products

The most frequently adulterated milk product is Ghee along with other milk products. The reason is the price of milk fat. It is commonly adulterated with animal body fats, vegetable oils and vanaspati.

8.6. Detection of Vanaspati in Ghee

Isolate the fat from milk by heat clarification method. Take about 5 g of the melted fat in a test tube. Add 5 ml of concentrated HCl (AR grade). Add 0.4 ml furfural solution (2% in alcohol) and shake the tube thoroughly for 2 min. Allow the mixture to separate. The development of pink or red color in the acid layer indicates presence of vanaspati. Confirm by adding 5 ml distilled water and shaking again. If the color in acid layer persists, vanaspati is present. If the color disappears, it is absent SP:18 (1987).

8.7. Detection of animal body fats and vegetable oils/fats by the Opacity Test in ghee

Melt the sample of fat (5 gm) isolated by heat clarification method at 50 +1°C in a test tube and maintain for 3 min to equilibrate. Then transfer the test tube at 23 + 0.2°C water bath and record the opacity time (Time taken by fat sample to acquire either O.D. at 570 nm between 0.14-0.16 or Klett reading using red filter between 58-62 after adjusting the instrument to 100% transmittance). The opacity time of pure buffalo ghee is 14-15 min, cow ghee is 18-19 min and that of ghee from cotton tract area is 11-12 min. The opacity time of buffalo ghee adulterated at 10% level with vanaspati is 10-11 min, with pig body fat is 8-9 min, with buffalo body fat is 2-3 min, with cow body fat is 3-4 min and with refined oils is 20-25 min (Singhal, 1980).

8.8 Test for Skimmed Milk Powder in Natural milk (Cow, Buffalo, Goat, Sheep)

Take 50 ml of milk in a 60 ml centrifuge tube. Place the tube in the centrifuge and balance it properly. Centrifuge at 3000 rpm for 15 minutes. Decant the supernatant creamy layer carefully. Add 0.5 ml of 4 % acetic acid for coagulation and then add 2 ml of 1 % phosphomolybdic acid in water. Mix the contents thoroughly and heat in a water bath at boiling temperature for 15 minutes and then cool. The curd obtained from pure milk shall be greenish in color whereas the curd of sample containing skimmed milk powder shall be bluish in color. The intensity of bluish color depends on the amount of the skim milk powder present in the sample.

8.8 Detection of Vegetable Oils in Milk Using Modified Gerber Butyrometer

Fig. 8.8 Modified gerber butyrometer

Isolate the fat from milk by Gerber method using specially designed milk butyrometer, which is open at both ends. Close the stem side opening with a good quality acid resistant silicon cork. Add 10 ml of 90% H_2SO_4, 10.75 ml milk and 1 ml amyl alcohol. Close the neck side with lock stopper; mix the contents and centrifuge for 5 min to get clear fat in the column. Remove the silicon cork and take out fat from the stem of butyrometer with the help of a capillary tube or a syringe. Place the fat on the prism of the butyrorefractometer maintained at 40°C and note down the reading. Since, B.R. reading is depressed due to hydrolytic effect of H_2SO_4 on the fat. Therefore, observed B.R. reading is corrected as follows

Corrected B.R. = 1.08 × Observed B.R.

The Corrected B.R. reading of milk fat thus obtained should be consistent with the values given for ghee as per PFA standards. Any deviation from the standard value indicates adulteration of milk with vegetable oils. However, this method has limitation of detection of adulteration with two oils i.e. coconut oil and palm oil whose values are close to that of milk fat (Arora et al, 1996).

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Lesson 9
ANALYSIS OF MARKET SAMPLES OF MILK AND MILK PRODUCTS

9.1 Introduction

Milk is an extremely complex biological fluid with scores of nutrients contained in a fluid with characteristics of three physical phases: a dilute emulsion, colloidal dispersion and a solution. The emulsion can be broken by low speed centrifugation and the milk separates into lipid and aqueous phases or compartments, each with a characteristic composition. The chemical make up of milk and its physico-chemical behavior provide scientific basis for process of milk and manufacture of products. Milk is valued commercially for two parameters namely fat and solids-not-fat (SNF). The SNF largely consists of proteins, lactose and minerals. The term total solid (TS) refers to the quantity of SNF plus fat present in milk. It may range from 12 to 16 percent, depending on its source. For cow milk, TS is 12 percent (3.5 % F and 8.5% SNF) while for buffalo milk it ranges between 15 and 16% (6-7% F and 9-10% SNF). Apart from species and breed related differences, certain other factors also influence the composition of milk. They are: individuality of animal, stage of milking, intervals of milking, completeness of milking, frequency of milking, irregularity of milking, portion of milking, different quarters of udder, lactation period, yield of milk, season, feed, nutritional level, environmental temperature, health status, age, weather, oestrum or heat gestation period, exercise, excitement and administration of drugs and hormones. In general, these variables tend to average out but show a seasonal pattern in the production of commercial milk used by dairy processors. This periodic fluctuation could have an important impact on properties of the finished products. However, variations in the concentration of an individual chemical entity are in certain ranges and the average values for these chemical constituents in milk provide useful information for several purposes:

- Nutritive values of milk and milk products
- Classification of milk as a basis for payment
- Detection of adulteration
- Breeding of milch animals for herd improvement
- Control of hygiene and health status of herd
- Production of specific product
- Processing effect on chemical quality
- Statutory requirement for meeting standards

So, for the above mentioned purposes, it is very necessary to go for the chemical analysis of milk and milk products. Before analysis, the sampling of milk and milk products shall be done by an experienced person as explained earlier in Lesson 7; Para 1.

9.2 Platform tests

Milk is a product of biological origin and is a suitable vehicle to accommodate any additive without apparent changes in its look. The quality of milk and its products depends upon quality of raw milk used in their manufacture, processing and handling conditions. It is therefore, necessary to check quality of raw milk to assess its suitability for processing, through various quick tests called “Platform Tests”. Moreover these tests are called platform tests because they are performed at the reception dock where milk is received and checked for its quality using various tests before either rejecting or accepting the milk supply for further processing.

9.2.1 Organoleptic test

Organoleptic test are used in all dairies and an experienced person can pick out bad samples with a high degree of accuracy. Judging the quality of milk by its taste and smell requires considerable skill which could only be acquired by practice.

9.2.1.1 Procedure

Remove lid from the can/tanker and observe for any extraneous matter present. Sniff the milk for any objectionable flavor that is un-natural of milk. Put small quantity (10 – 20 ml) on tongue and roll into mouth cavity for any off taste that is un-natural of milk and spill out. In case of doubt, subject the sample to other tests. Based on organoleptic evaluation accept or reject the milk.

9.2.2 Clot-on-boiling test (COB)

This is a quick test to determine developed acidity and to assess the suitability of milk for heat processing.

9.2.2.1 Procedure

Take 5.0 ml of milk in a 20 ml test tube using graduated pipette. Place the tube in boiling water bath for five minutes or hold the tube over a flame and allow the contents to boil. Formation of clots or flakes on the test tube wall indicates positive test. This further indicates that the milk has high developed acidity and is not suitable for heat processing.

9.2.3 Alcohol test

The alcohol test is used for rapid assessment of stability of milk to processing, particularly for condensing and sterilization. This test gives an indication of the mineral balance of milk. The test aids in detecting abnormal milk such as colostrum, milk from the animal in the late lactation, milk from animal suffering from mastitis and milk in which mineral balance has been disturbed.

9.2.3.1 Procedure

Take 5.0 ml of milk in a test tube using graduated pipette. Add equal amount of ethyl alcohol (75% ethyl alcohol for cow milk and 68% ethyl alcohol for buffalo milk). Close the mouth of the test tube with thumb and mix the contents well by inverting the test tube several times. Formation of any flakes on the wall of the test tube indicates positive test.

9.2.4 Alcohol –alizarin test

The test is similar to the alcohol test and the incorporation of Alizarin helps to indicate the approximate percentage of acidity. So this test also indicates the suitability of milk for high heat treatment and to have idea about milk acidity without acidity test.

9.2.4.1 Procedure

Transfer 5.0 ml of milk in a test tube using graduated pipette. Add equal amount of alcohol-alizarine solution (0.2%).Mix the contents well by inverting the tube several times. Observe for formation of flakes and color of the contents. Match your results with following table

Table 9.1 Alcohol-alizarin test observations

Range of color	Presence of flakes	Approximate Acidity (%)
Pale red	Nil/no	0.16
Reddish brown	Small	0.20
Yellowish brown	Small	0.24
Brownish yellow	Large	0.28
Yellow	Large	0.36

9.2.4.1 Detection of neutralizers

Alkali in various forms like sodium carbonate, sodium bicarbonate, sodium hydroxide and lime are used to neutralize developed acidity in milk. Detection of such neutralizers can be made by the following two tests. For details, refer Lesson 8.

9.3 Laboratory Tests

9.3.1 Acidity test

Normal acidity of milk is due to its constituents like casein, citrates, phosphates and CO_2. This acidity can be measured by titrating milk against a standard alkali solution using an indicator and is expressed in terms of lactic acid. The aim of the test is to assess suitability of milk for heat processing.

9.3.1.1 Requirements

Pipette, porceline dish, glass stirring rod, burette (50 x 0.1 ml), Sodium hydroxide solution (N/10), phenolphthalein solution (0.5%)

9.3.1.2 Procedure

Transfer 10 ml of milk into a white porcelain dish, with the help of pipette add 1.0 ml of 0.5% phenolphthalein solution as indicator. Titrate the contents with N/10 NaOH solution. Observe occurrence of pink color as end point for the titration. Note the titre value. Calculate percent acidity of the sample as lactic acid.

9.3.1.3 Calculation

$$\text{Titrable acidity \% (as lactic acid)} = \frac{9 \times V_1 \times N}{V_2}$$

Where, V_1 = Volume of N/10 NaOH used
V_2 = Volume of milk sample
N = Normality of NaOH used

9.4 Determination of Fat in Milk and Milk Products

Fat is the most important and valuable constituent of milk and milk products. It also plays an important role in the pricing of milk and milk products. Estimation of fat in milk can be done in two ways:

1. Gerber method
2. Gravimetric method (using Rose-Gottlieb method)

9.4.1 Gerber method

9.4.1.1 Determination of fat in milk

Principle: When a definite quantity of sulphuric acid and amyl alcohol are added to a definite volume of milk, the proteins will be dissolved and the fat will be set free which remains in liquid state due to heat produced by the acid. The amyl alcohol used facilitates the separation of a clear fat column. On centrifugation, fat being lighter will be separated on top of the solution.

a) Requirements

Gerber centrifuge, Gerber butyrometers for milk (0-10% scale with 0.1 per cent mark). Hot water bath maintained at 65°C ±2°C. 10 ml automatic measure for acid, 1 ml automatic measure for amyl alcohol, 10.75 ml milk pipette, butyrometer stoppers, butyrometer stand, key for stoppers, Gerber sulphuric acid density 1.807 to 1.812 g/ml at 27°C corresponding with a concentration of sulphuric acid from 90 to 91% by weight. Iso- amyl alcohol 95% of clear, colorless liquid and shall distil between 130°C to 132°C having density 0.803 to 0.805 g/ml at 27°C.

b) Procedure

Take 10 ml of Gerber sulphuric acid from automatic measure into the butyrometer. Pipette out 10.75 ml of the well mixed sample of milk and transfer it to the butyrometer carefully without allowing it to mix with the acid. This is done by allowing the jet of milk from the pipette to hit the inside wall of the butyrometer by holding the pipette in a slanting manner and resting the tip end on the mouth of the butyrometer. With the help of automatic pipette add 1 ml iso - amyl alcohol to the above butyrometer. Insert the stopper with the help of key and tight the stopper and mix the content by shaking the butyrometer at a 45 degree angle until all the curd has been dissolved. Keep the butyrometer in the water bath at 65°C ± 2°C for 5 minutes. Place the butyrometer in the centrifuge and balance the machine. Centrifuge for 5 minutes, (1000-1200 rpm). After centrifuging, temper the butyrometer in the water bath at 65°C ± 2°C for 5 minutes. With the help of key, adjust the fat column within the scale on butyrometer and take the reading and record the fat percentage. Care must be taken not to wet the neck of the butyrometer while adding Gerber sulphuric acid, milk and amyl alcohol. The test must be repeated if particles of curd are observed.

9.4.1.2 Determination of fat in cream

a) Apparatus

As in the case of milk except cream butyrometer (0- 70%) and a physical balance

b) Reagents

Fifty per cent Gerber sulphuric acid prepared by mixing equal volumes of sulphuric acid and

distilled water at the time of experiment. Iso - amyl alcohol as in the case of milk.

c) Principle

Same as milk

d) Procedure

Stir the sample by carefully without causing frothing or churning. If the cream is very thick warm to 30°C-40°C to facilitate mixing. Immediately before weighing mix the sample. Keep the cream butyrometer with a small funnel at the mouth in a convenient conical flask and weight it. Then weigh accurately 5.00 ± 0.01 g of the cream into it. Add small quantities of the freshly prepared acid to the funnel to ensure complete washing down of the cream to the butyrometer. Add About 18-20 ml of the dilute acid to the butyrometer leaving sufficient space for the addition of amyl alcohol. Add one ml of amyl alcohol and proceed as in case of milk.

9.4.1.3 Determination of fat in Dahi

a) Apparatus

Same as given under milk

b) Reagents

Same as for milk

c) Principle

It is same as for milk. In addition, ammonia is added to liquefy the curd particles and to make the sample homogeneous.

d) Procedure

Weigh 100 g of the well mixed dahi sample in beaker. Add 5 ml of strong ammonia to the weighed sample and shake well to make it homogenous. Take the above prepared sample and proceed as in the case of milk. Multiply the result obtained by the dilution factor (in this case 105/100) and add the same to the obtained result to get the actual result.

9.4.1.4 Determination of fat in cheese

a) Apparatus

Gerber centrifuge; Cheese butyrometer (0- 40%), tilt measure of 10 ml and 1 ml capacity

Fig. 9.1 Tilt measure

b) Reagents

Gerber sulphuric acid (sp. gr. 1.820 at 15.6°C); iso-amyl alcohol

c) Principle

Same as in milk

d) Procedure

Weigh accurately about 3 ± 0.01 g of cheese sample into the cheese butyrometer. Add 10 ml of warm distilled water (30°C -40°C). Add 10 ml of Gerber sulphuric acid into the butyrometer. Add 1 ml of iso-amyl alcohol. Close the butyrometer with the stopper and shake well till all the contents are well mixed. Place the butyrometer in a water bath at 65°C ± 2°C for tempering. Shake periodically until the solution of cheese is complete. Centrifuge at 1200 rpm for 5 minutes. Read the percentage of fat by adjusting the fat column within the scale of the butyrometer.

9.4.1.5 Determination of fat in butter

a) Apparatus

As in the case of milk except butter butyrometer (0- 90%) and a physical balance

b) Reagents

Fifty per cent Gerber sulphuric acid prepared by mixing equal volumes of sulphuric acid and distilled water at the time of experiment. Add iso-amyl alcohol as in the case of milk.

c) Principle

Same as milk

d) Procedure

Weigh accurately about 5 ± 0.01g of butter sample in 25 ml beaker and mix it with small portion of 1:1 sulphuric acid. Transfer the contents into the butter butyrometer. Dilute sulphuric acid with distill water and add 15 to 20 ml in butyrometer. Add 1 ml of isoamyl alcohol. Rest of the method is same as in case of milk.

9.4.2 Rose-Gottlieb method

This method of fat estimation is used as a reference method. The method is a gravimetric in nature unlike Gerber method which is a volumetric method and is followed for routine purposes.

9.4.2.1 Determination of fat in milk and milk product

a) Apparatus

Fat extraction apparatus (Rose-Gottlieb tube), Oven at 100 ± 2°C, Water bath boiling.

b) Reagents

Concentrated ammonia solution, concentrated HCl, Ethyl alcohol, Diethyl ether (solvent ether), Petroleum ether (40-60°C).

c) Principle

For quantitative separation of fat from milk and milk products, it is necessary to break up the protective film surrounding the fat globule by using suitable agents (ammonia in case of liquid milk, and concentrated HCl in case of concentrated /dried milk products).
Ammonia/Concentrated HCl brings about the break up of the protective layer. Ethyl alcohol facilitates the passage of fat from the aqueous phase to the solvents. The fat after extraction gets dissolved in the mixture of solvents (diethyl ether and pet. ether)The fat dissolved in the solvent mixture is collected in a tared conical flask containing 2-3 glass beads and then the solvents are evaporated on the water-bath and the finally dried in the oven. The weight of the dried fat in the flask is taken and percentage in the product is calculated.

d) Procedure

Weigh accurately a known quantity of well mixed sample (for example, 10 gm of liquid milk or 1 gm of concentrated/dried milk product) into the fat extraction (Rose-Gottlieb) tube. Add 1 ml conc. ammonia in case of liquid milk or 10 ml of concentrated HCl in case of concentrated/dried milk products and mix well. In case of concentrated dried milk products, heating is done on a water bath till casein has dissolved, then contents are cooled. Add 10 ml of ethyl alcohol and again mix well. Add 25 ml of diethyl ether (solvent ether) and mix properly. Then add 25 ml of petroleum ether (40-60°C) and again mix properly. Allow the extraction tube to stand till the two layers are separated clearly (Approx. 30 minutes). Siphon off the ether solution i.e. upper layer into the tared conical flask containing 2-3 glass beads. Repeat the process of extraction at least twice using 15 ml. of solvent ether and 15 ml of petroleum ether, and similarly pour these two extracts into the same tared conical flask. Evaporate carefully the solvents from the flask over a boiling water bath. Dry the residual fat in the oven at 100 ± 2°C for 1-2 hours. Cool the flask to room temperature in a desiccator and weigh it. Repeat the process of heating, cooling and weighing till you get a constant weight.

e) Observations

Weight of sample taken = W gm
Weight of empty tared flask with glassbeads = W_1 gm
Weight of empty tared flask with glassbeads + fat after drying = W_2 gm
Weight of fat in the flask = W_2 - W_1 gm = X gm.

f) Calculations

Percent fat in the milk or milk product = X/W× 100

Precautions: Solvents like Diethyl ether, Petroleum ether and ammonia should be stored at low temperature before opening the bottles. There should not be any naked flame near the place of experiment, since solvents used in this experiment are highly inflammable.

9.5 Determination of Solids-Not-Fat Content (SNF test) in Milk

9.5.1 Solids-not-fat content (SNF test) by lactometer

Lactometers are used for rapid determination of specific gravity of liquids. The method is based on the law of floatation, which states that when a solid is immersed in a liquid it is subjected to upward thrust equal to the weight of liquid displaced by it and acting vertically upwards. Lactometers are variable immersion type hydrometers and calibrated before hand with liquid of known specific gravity.

a) Requirements

Lactometer calibrated at 27°C (BIS), lactometer jar, thermometer.

b)Procedure

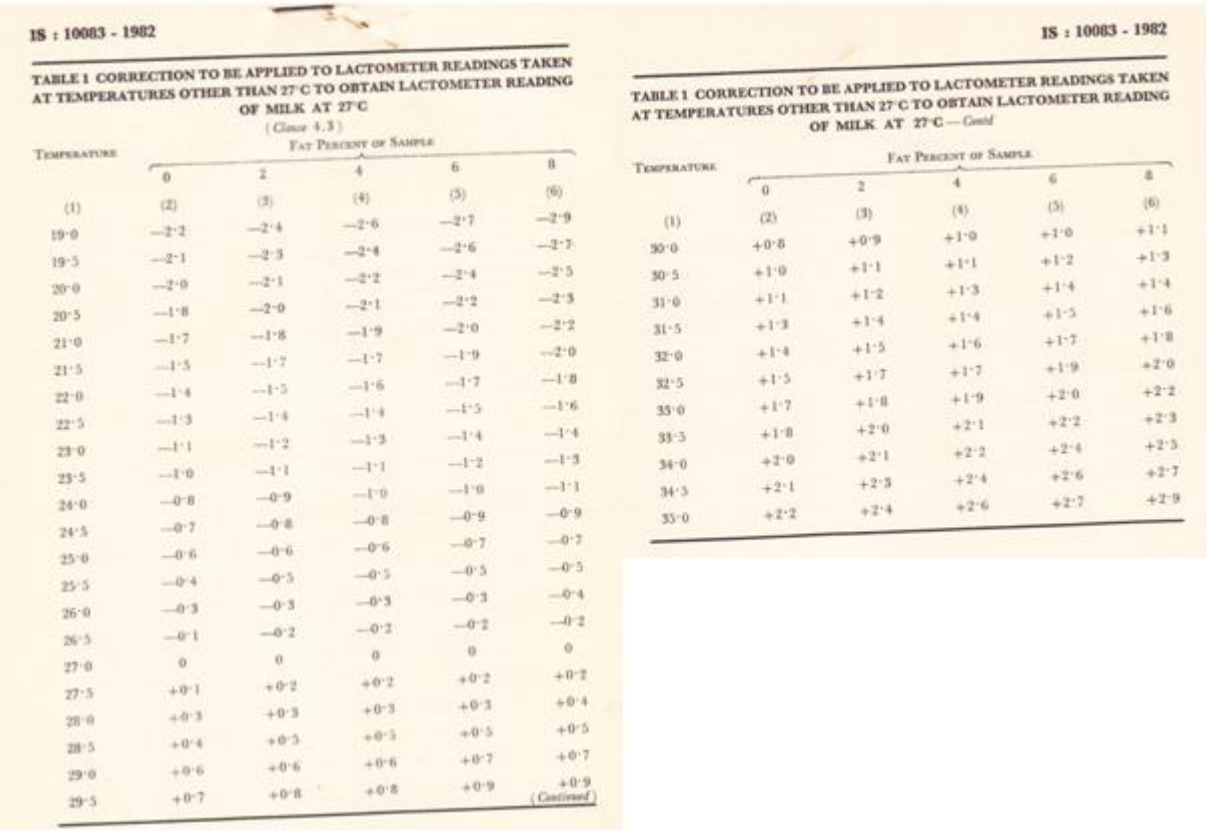
Adjust the temperature of milk sample to measuring temperature prescribed for lactometer (27°C). Mix the sample well to avoid incorporation of air or foam formation. Pour sufficient milk into the glass or steel cylinder to allow free floating of lactometer. Place the lactometer in the milk and allow it to float till it stops and assumes a constant level. Record the lactometer reading and temperature of milk at the same time. Take another reading by flapping the top of the lactometer stem and when it again assumes constant level. Take average of the two readings. Get corrected lactometer reading (CLR) from the standard table for corresponding temperature. Calculate solids-not-fat (SNF) content using the given formulas.

c) Calculations

Percent SNF=CLR/4+0.25 F+0.44

Where; F= Fat percentage in milk sample.

Correction factor for BIS lactometer



IS : 10083 - 1982

TABLE 1 CORRECTION TO BE APPLIED TO LACTOMETER READINGS TAKEN AT TEMPERATURES OTHER THAN 27°C TO OBTAIN LACTOMETER READING OF MILK AT 27°C

(Clause 4.3)

Temperature	Fat Percent of Sample				
	0	2	4	6	8
(1)	(2)	(3)	(4)	(5)	(6)
19·0	−2·2	−2·4	−2·6	−2·7	−2·9
19·5	−2·1	−2·3	−2·4	−2·6	−2·7
20·0	−2·0	−2·1	−2·2	−2·4	−2·5
20·5	−1·8	−2·0	−2·1	−2·2	−2·3
21·0	−1·7	−1·8	−1·9	−2·0	−2·2
21·5	−1·5	−1·7	−1·7	−1·9	−2·0
22·0	−1·4	−1·5	−1·6	−1·7	−1·8
22·5	−1·3	−1·4	−1·4	−1·5	−1·6
23·0	−1·1	−1·2	−1·3	−1·4	−1·4
23·5	−1·0	−1·1	−1·1	−1·2	−1·3
24·0	−0·8	−0·9	−1·0	−1·0	−1·1
24·5	−0·7	−0·8	−0·8	−0·9	−0·9
25·0	−0·6	−0·6	−0·6	−0·7	−0·7
25·5	−0·4	−0·5	−0·5	−0·5	−0·5
26·0	−0·3	−0·3	−0·3	−0·3	−0·4
26·5	−0·1	−0·2	−0·2	−0·2	−0·2
27·0	0	0	0	0	0
27·5	+0·1	+0·2	+0·2	+0·2	+0·2
28·0	+0·3	+0·3	+0·3	+0·3	+0·4
28·5	+0·4	+0·5	+0·5	+0·5	+0·5
29·0	+0·6	+0·6	+0·6	+0·7	+0·7
29·5	+0·7	+0·8	+0·8	+0·9	+0·9

(Continued)

IS : 10083 - 1982

TABLE 1 CORRECTION TO BE APPLIED TO LACTOMETER READINGS TAKEN AT TEMPERATURES OTHER THAN 27°C TO OBTAIN LACTOMETER READING OF MILK AT 27°C — Contd

Temperature	Fat Percent of Sample				
	0	2	4	6	8
(1)	(2)	(3)	(4)	(5)	(6)
30·0	+0·8	+0·9	+1·0	+1·0	+1·1
30·5	+1·0	+1·1	+1·1	+1·2	+1·3
31·0	+1·1	+1·2	+1·3	+1·4	+1·4
31·5	+1·3	+1·4	+1·4	+1·5	+1·6
32·0	+1·4	+1·5	+1·6	+1·7	+1·8
32·5	+1·5	+1·7	+1·7	+1·9	+2·0
33·0	+1·7	+1·8	+1·9	+2·0	+2·2
33·5	+1·8	+2·0	+2·1	+2·2	+2·3
34·0	+2·0	+2·1	+2·2	+2·4	+2·5
34·5	+2·1	+2·3	+2·4	+2·6	+2·7
35·0	+2·2	+2·4	+2·6	+2·7	+2·9

9.5.2 Total solid content of milk by gravimetric method

Whole milk contains about 84 –87% water and remaining 13-16% are solids comprising of fat, protein, carbohydrate and minerals. By evaporating water from the milk under controlled conditions the total solid content can be determined accurately.

a) Requirements

Aluminium moisture dish, boiling water bath/hot plate, weighing balance, hot air oven, desiccators, tong.

b) Procedure

Weigh accurately a clean and dry empty dish (A). Transfer 5.0 ml of sample into the dish and note the weight (B). Place the dish on boiling water bath or hot plate for 20-30 minutes. Allow the water to evaporate. Place the dish in hot air oven at 100 ± 1°C for 3 hours. Transfer the dish to a desiccator and allow cooling for 30 minutes. Weigh the contents and note the weight (C). Repeat heating and cooling until difference in two successive weights do not exceed 0.5 mg.

c) Calculations

Weight of sample (X) = Reading B – Reading A

Weight of solids in milk (Y) = Reading C – Reading A

$$\text{Percent ash by weight} = \frac{B}{A \times 100}$$

9.6 Determination of Lactose Contents in Milk and Milk Products

The lactose in milk and milk products may be quantified by methods based on one of the five principles namely polarimetry, oxidation-reduction titration (Lane- Eynon method), colorimetry, chromatography and enzymatically. Polarimetric method is simple, accurate and rapid for the estimation of lactose in milk and milk products. However, Lane- Eynon method is most commnly used and widely accepted method.

9.6.1 Lane-Eynon method

9.6.1.1 Principle

Reducing sugars, which are more common, are able to function as reducing agents because of free aldehydic/ketonic groups present in the molecule. The reducing properties of these sugars are usually observed by their ability to reduce metal ions notably copper, iron and silver in alkaline solution. Those very properties of sugars have been used in this method. The reducing sugar solution reduces an alkaline cupric-salt solution during boiling and converts into the red cuprous-oxide. From the reduced amount of copper salt, the quantity of reducing sugar is estimated. Fehling solution is a mixture of Fehling A ($CuSO_4.H_2O$) and Fehling B (alkaline sodium potassium tartarate). When copper sulphate is made alkaline, it gives the blue colored precipitates of Cu $(OH)_2$. But the presence of sodium potassium tartarate forms a soluble blue colored complex of copper compound behaves as if it is alkaline Cu $(OH)_2$ solution. In general, when NaOH is added $CuSO_4$, a blue precipitate of Cu $(OH)_2$ is obtained (Fehling A). These precipitates are made to dissolve in Rochelle salt solution (Fehling B) and a blue color solution is obtained, which is known as Fehling solution. The Fehling solution when heated gives rise to cupric oxide (CuO) which in turn reacts with reducing sugar and gets reduced to cuprous oxide (Cu_2O) brick red precipitate and resulting into the oxidation of sugars to corresponding acids.

9.6.1.2 Reactions

$CuSO_4 + 2NaOH \rightarrow Na_2SO_4 + Cu(OH)_2$
$RCHO + 2Cu(OH)_2 \rightarrow RCOOH + 2H_2O + Cu_2O\downarrow$

9.6.1.3 Reagents

a) Fehling solution A

Weigh 34.639 gm $CuSO_4.5H_2O$ and dissolve in distilled water. Add 0.5 ml of Concentrated H_2SO_4 and make the volume to 500 ml.

b) Fehling solution B

Weigh 173 gm Rochelle salt and 50 gm NaOH and dissolve in distilled water and make the volume to 500 ml.

c) Zinc acetate solution (2N)

Dissolve 21.9 gm of crystallized zinc acetate [$Zn(C_2H_3O_2).H_2O$] in distilled water add 3 ml of acetic acid and make up the volume to 100 ml.

d) Potassium Ferrocyanide (1N)

Dissolve 10.6 gm Potassium Ferrocyanide, $K_4Fe(CN)_6.3H_2O$ in distilled water and make upto 100 ml.

e) Acetic acid solution

Equivalent to dilute NH_3 strength.

f) Dil. aqueous ammonia solution (10%)

10 ml Conc.NH_3, diluted to 100 ml.

g) Aqueous methylene blue indicator 0.2%

Dissolve 0.2 g of methylene blue in distilled water and make the volume to 100 ml.

h)Standard lactose solution (5%)

Dissolve 5 gm lactose in distilled water and make the volume to 100 ml.

9.6.1.4 Procedure

a) Standardization of Fehling solution

Pipette out 5 ml of Fehling solution A and 5 ml of Fehling solution B in a 100-150 ml conical flask and mix. Take the standard lactose solution in a burette. Warm the contents in a conical flask over the burner and add a little less than expected amount of sugar solution and allow it to boil. Add a few drops of methylene blue. Now add drop wise the sugar solution from the burette till the blue color disappears and brick red color appears. By this method, determine the actual amount of standard solution utilized against 5 ml of aqueous solution.

b) Sample preparation

Weigh accurately 40 gm of sample in a 100 ml beaker, to that add 50 ml of hot water (30-90°C). Mix well. Transfer the contents to a 250 ml volumetric flask and rinse the beaker with hot water to make the volume to about 120-150 ml. Add 5ml of 10% dil. NH3 solution, mix well for 10-15min. Add 5 ml of 10% acetic acid to neutralize ammonia (equivalent amount of glacial acetic acid). Add 12.5 ml of zinc acetate solution and 12.5 ml of Potassium Ferrocyanide solution to precipitate the proteins and mix well. Make up the volume to 250ml with distilled water. Filter through Whatman No.1. Discard some amount of filtrate and the rest is used for the titration.

c) Estimation of reducing sugar

Take 25 ml of this sample solution (filtrate), dilute it to 100 ml and titrate against 10 ml Fehling solution (5 ml Fehling A + 5 ml Fehling B).

d) Calculation

10 ml Fehling (A+B) solution = V_1 ml of standard lactose sugar solution of concentrated C_1 mg/l

10 ml Fehling (A+B) solution = V_2 ml of sample solution of Concentration C_2

$$V_1C_1 = V_2C_2$$

Therefore,

$$C_2 = \frac{V_1C_1}{V_2} \text{mg/ml}$$

$$C_2(\text{lactose}) = \frac{V_1C_1 \times 250 \times 100}{V_2 \times 1000 \times 40 \times 25} \times 100\%$$

9.7 Determination of Protein Contents in Milk and Milk Products by IDF and BIS Methods

9.7.1 Principle

The test portion is digested using a block-digestion or equivalent apparatus with a mixture of concentrated sulfuric acid and potassium sulfate, using copper (II) sulfate as a catalyst to convert organic nitrogen to ammonium sulfate. Addition of excess sodium hydroxide to the cooled digest liberates ammonia. The liberated ammonia is distilled, using either a manual or semi-automatic steam distillation and collected into an excess of boric acid solution followed by titration with hydrochloric acid solution. The nitrogen content is calculated from the volume of HCl used by ammonia in the titration and multiplied by 6.38 to get the corresponding crude protein content.

9.7.2 Chemistry of nitrogen determination

A. Solubilization stage

$$\text{Proteins} + H_2SO_4 + Na_2SO_4 \text{ or } K_2SO_4 \xrightarrow{Cu^{2+}} (NH_4)_2SO_4$$

B. Release of ammonia and steam distillation

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_4OH + Na_2SO_4$$

$$NH_4OH \xrightarrow{\text{Steam}} NH_3\uparrow + H_2O\uparrow$$

C. Collection of ammonia and back titration

$$NH_3 + H_2O + H_3BO_3 \longrightarrow NH_4H_2BO_3$$

$$NH_4H_2BO_3 + HCl \longrightarrow NH_4Cl + H_3BO_3$$

9.7.3 Conversion factor

Proteins in general vary in nitrogen content from 14 to 19% and thus a single universal conversion factor cannot be used. An average factor of 6.38 (corresponding to 15.65% N) is commonly used for milk proteins to convert nitrogen to protein.

9.7.4 Sample size

The protein content of different types of milk and milk products varies so for determination of total protein content by Kjeldahl method the size of sample varies such that the test portion sample should contain 0.2 to 0.4 g of protein.

9.7.5 Apparatus

Water bath; Analytical balance; Digestion block; Digestion tube, 250 ml capacity; Exhaust manifold; Aspirator; Automatic pipettes (dispensers); Graduated measuring cylinder 50 ml capacity; Distillation unit; Conical flask 250 ml capacity; Burette 25 ml capacity.

9.7.6 Reagents

- Kjeldahl catalyst mixture (Digestion mixture): It consists of 3.5 g potassium sulfate and 0.105 g copper sulfate.
- Sulfuric acid: with a mass fraction of at least 98% nitrogen free.
- Sodium hydroxide solution: Nitrogen free, containing approximately 40 g sodium hydroxide per 100 ml.
- Boric acid solution: Dissolve 40 g of boric acid in 1 litre of hot water in a 1000 ml one-mark volumetric flask. Allow the contents to cool to 20°C and adjust the mark with water.
- Indicator solution: Dissolve 0.25 g of methylene blue and 0.375 g of methyl red in 300 ml of 95 per cent ethanol.
- Hydrochloric acid 0.1N
- Tryptophan or lysine hydrochloride, minimum assay 99% (mass fraction).
- Sucrose, with nitrogen content not more than 0.002%.

9.7.7 Procedure

9.7.7.1 Preparation of test sample

Warm the test sample to between 38°C to 40°C in the water bath. Cool the sample to room temperature, while gently mixing the test sample immediately prior to weighing the test portion.

9.7.7.2 Test portion and pre-treatment

To a clean and dry digestion tube, add 5 g of digestion mixture. Weigh 2 g of test sample to the nearest 0.1 mg into the tube. Carefully add 10 ml of sulfuric acid along the sides of the digestion tube. Gently mix the contents of the tube and then leave to stand for 10 min.

9.7.7.3 Digestion

Fig. 9.2 Digestion Block

Set the digestion block at a low initial temperature so as to control foaming (approximately 180°C). Transfer the tubes to the digestion block and place the exhaust manifold which is itself connected to a water jet pump in the top of the tube. The suction rate of the water jet pump should be just sufficient to remove fumes. Digest the sample until white fumes develop. Then increase the temperature of digestion block to between 410°C and 430°C and continue digestion of the sample until the digest is clear. After the digest clears (clear with light blue-green color), continue digestion at between 410°C and 430°C for at least 1 h. During this time the sulfuric acid should be boiling. If visible boiling of the clear liquid is not apparent as bubbles forming at the surface of the hot liquid around the perimeter of the tube, then the temperature of the block may be too low. The total digestion time will be between 1.75 h and 2.5 h. At the end of the digestion, the digest shall be clear and free from undigested material. Remove the tube from the block with the exhaust manifold in place. Allow the digest to cool to room temperature over a period of approximately 30 min. The cooled digest should be liquid with a few small crystals at the bottom of the tube. Excessive crystallization indicates too little residual sulfuric acid at end of the digestion and may cause a decrease in protein estimation results. To reduce acid loss during digestion, reduce aspiration rate. After the digest has cooled to room temperature in approx. 30 min, remove the exhaust manifold and carefully add 50 ml of water to each tube. Swirl to mix while ensuring that any separated out crystals are dissolved. Allow the contents of the tube to cool to room temperature again.

9.7.7.4 Distillation

Fig. 9.3 Distillation unit

Transfer the digestion tube to the distillation unit and place a conical flask containing 50 ml of boric acid solution under the outlet of condenser in such a way that the delivery tube is below the surface of the excess boric acid solution and run the programme for automatic distillation Adjust the distillation unit to dispense 60 ml of sodium hydroxide solution and distill off the ammonia liberated by the addition of sodium hydroxide solution. Following the manufacture's instructions, operate the distillation unit in such a way as to steam distil the ammonia liberated by addition of sodium hydroxide solution, collecting the distillate in the boric acid solution containing mixed indicator. Continue with the distillation process until at least 150 ml of distillate is collected. Remove the conical flask from the distillation unit and completely drain the distillation tip. Rinse the inside and outside of the tip with water, collecting the rinsing in the conical flask. Always rinse the tip with water between samples.

9.7.7.5 Titration

Titrate the contents of the conical flask with the 0.1N hydrochloric acid using a burette and read out the amount of titrant used. The end point is reached at the first appearance of violet color in the contents.

9.7.7.6 Blank test

Carry out a blank test following the procedure described above taking 5 ml of water and about 0.85 g of sucrose instead of test portion.

9.7.7.7 Recovery tests

The accuracy of the procedure should be checked regularly by means of recovery tests as given below:

a) Check that digestion and distillation procedures are efficient by using a test portion of 0.06 g of lysine hydrochloride or 0.08 g of tryptophan weighed to the nearest 0.1 mg.

b) Determine the nitrogen content according to the procedure described above (9.7.7). The expected nitrogen content is 15.33% in lysine and 13.72% in tryptophan (the nitrogen recovery should be (98.5% to 101%).

c) Prepare a solution of ammonium sulphate of concentration 0.05 mol/L exactly. Pipette a 10 ml aliquot of the ammonium sulphate solution into the digestion tube and add 50 ml of water. Determine the nitrogen content of the solution according to the procedure described in 9.7.7 (nitrogen recovery should be 99% to 101%).

9.7.7.8 Calculation

Calculate the nitrogen content, Wn, by using the following equation:

$$W_n = \frac{1.4\,(V_s - V_b)\;N}{m}$$

Where,

Wn = is the nitrogen content of the sample, expressed as percentage by mass
Vs = is the numerical value of hydrochloric acid solution used in determination in millilitres, expressed to the nearest 0.05 ml
Vb = is the numerical value of the volume of hydrochloric acid solution used in the blank test in millilitres, expressed to the nearest 0.05 ml
N = is the numerical value of the exact normality of the hydrochloric acid solution expressed to four decimal places.
m = is the numerical value of the mass of the test portion in grams, expressed to the nearest 1 mg, Calculate the crude protein content Wp using the following equation:

Wp = Wn x 6.38

where, Wp = is the crude protein content, expressed as a percentage by mass.
Wn = is the nitrogen content of the sample, expressed as a percentage by mass to four decimal places.
= is the generally accepted multiplication factor to express the nitrogen content as crude protein content

9.7.8 BIS method

9.7.8.1 Apparatus

i) Digestion flask – Kjeldahl flask

ii) Distillation apparatus – Micro Kjeldahl distillation

The micro Kjeldahl apparatus for steam distillation is shown above. Steam is produced by boiling water in flask A, and this is bubbled through the solution in flask C. The distillate passes through the condenser D and can be collected at the end of the condenser. A trap B is provided between flasks A and C in such a way, that when A is cooled, the contents in C are sucked out into flask B which can then be discarded. There is also a funnel arrangement F provided to add samples directly to flask C.

9.7.8.2 Reagents

a) Concentrated sulfuric acid – approx. 98% by weight and nitrogen free (ρ=1.84 g/cm^3)
b) Copper sulphate
c) Potassium sulphate or anhydrous sodium sulphate (nitrogen free)
d) Sodium hydroxide solution – 50% by wt.
e) Boric acid solution – saturated
f) Indicator solution – Mix equal volumes of a saturated solution of methyl red in ethanol (95% by vol.) and a 0.1% solution of methylene blue in ethanol (95% by vol.)
g) Standard hydrochloric acid – 0.02N

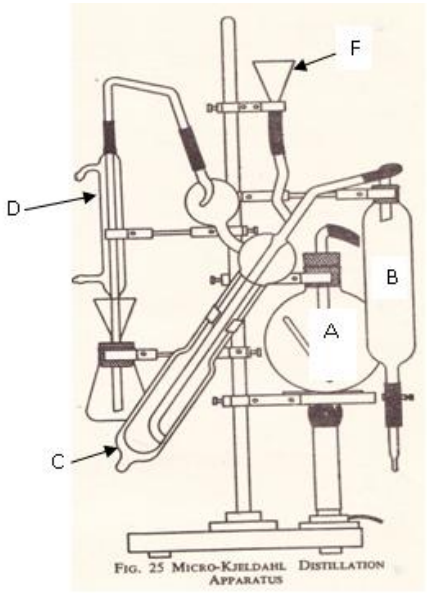


Fig. 9.4 Micro Kjeldahl distillation apparatus

9.7.8.3 Preparation of test sample

Warm the test sample of milk between 38°C to 40°C in the water bath. Cool it to room temperature while gently mixing the test sample immediately prior to weighing the test portion.

9.7.8.4 Procedure

a) Digestion of sample

Transfer accurately weighed (approx.) 10 g sample of milk to a Kjeldahl flask. Add 10 g of potassium sulphate and 0.2 g copper sulphate. Add 25 ml of concentrated sulfuric acid, along the neck of the flask in such a way as to wash down any milk drops sticking to the side of the flask. Gently rotate the flask so that the whole of the contents are well mixed. Place the flask on a flame so that the neck is inclined at an angle of 45° to the horizontal and the bulb rests in the hole of an asbestos sheet so that the flame does not touch the flask above the level of the liquid. Heat initially to gentle boiling and when frothing has ceased, boil the contents of the flask briskly until clear and free from yellowish color and for a further period of one hour. Allow the liquid to cool and wash down the sides with a fine jet of distilled water. Continue heating the contents of the flask for a further period of one hour. Allow the liquid to cool to room temperature and make upto volume in a 100 ml volumetric flask.

b) Distillation

Pipette out 10 ml aliquot of the solution into the flask 'C' through the funnel of micro Kjeldahl distillation apparatus. Then add 8 ml of sodium hydroxide solution through funnel. Keep a flask containing 10ml of the boric acid solution containing 2-3 drops of the indicator, at the delivery end of the condenser in such a way that the tip is just beneath the surface of the liquid. Now heat the flask 'A' filled with water to produce steam. This steam is passed through the contents of flask 'C'. The ammonia evolved there by the alkaline treatment of digested sample is carried along with steam through the condenser outlet and is absorbed in boric acid solution. Continue passing steam for 10 minutes and collect about 50 ml of distillate, then remove the receiver flask after rinsing out the tip of the condenser. Stop heating flask 'A'. On cooling, this will create a back suction so that the contents in the flask 'C' will be sucked into the trap 'B'. Add about 10 ml of water through the funnel quickly, so that it will also be sucked into flask 'B', while rinsing flask 'C'. The apparatus is now ready for distillation of next sample. Titrate the contents in the receiver flask against the standard hydrochloric acid till the color changes from green to violet color. Note the volume of acid used. Carry out a blank determination by taking 0.5 g of sucrose in place of milk, and by using the same quantities of reagents and the same conditions of test.

c) Calculations

Crude protein is calculated by multiplying nitrogen content by the factor 6.38.

$$\text{Crude protein (percent by wt.)} = \frac{6.38 \times 1.4\ (V2 - V1)\ N \times \text{DF}\ (100/10)}{W}$$

Where,

V_2 = Number of ml of hydrochloric acid standard volumetric solution used in distillation
V_1 = Number of ml of hydrochloric acid standard volumetric solution used in the blank test
N = Normality of hydrochloric acid standard volumetric solution
W = Weight in g of the sample taken for analysis
DF = dilution factor

9.8 Total ash content

9.8.1 Principle

Milk contains soluble salts like sodium, potassium, calcium, phosphorus, citrates, sulphates, chlorides, carbonates, magnesium etc. Heating milk at higher temperatures decomposes organic matter and the soluble inorganic salts are left in the form of ash.

9.8.2 Requirements

Single pan balance, muffle furnace, desiccator, silica crucible, tong, hot plate.

9.8.3 Procedure

Accurately weigh 10 g of milk sample in to the silica crucible. Evaporate the sample to dryness on a hot plate. Place the crucible in a pre-heated muffle furnace and heat the contents at 550°C until ash is free from carbon. Cool the crucible by placing in desiccator. Weight the crucible containing the ash. Continue heating, cooling and weighing until the difference in weight is not more than 0.1 gm.

9.8.4 Calculations

weight of empty crucible = W g
weight of crucible with milk = W_1 g
weight of crucible after drying = W_2 g
weight of milk sample = W_1– W g (A)
weight of ash = W_2 – W g (B)

$$\text{Percent ash by weight} = \frac{B}{A \times 100}$$

Lesson 10
INSTRUMENTAL METHODS OF ANALYSIS

10.1 Introduction

The technology of using instruments to measure and control the physical and chemical properties of material is called instrumentation. Investigations in food science and technology, whether in universities, governmental agencies, or the food industry, often require determination of food composition. The dairy scientists and technologists often determine the chemical composition as part of research on food product development or quality assurance activities. The chemical composition of foods is often determined to establish the acceptability or nutritive value of food product. The field of milk and milk product analysis involves a considerable amount of time spent learning principles, methods and instrument operations and perfecting various techniques. Also the relationship between the physical properties of milk and its chemical composition has been exploited in the dairy industry to develop various instrumental methods for the determination of chemical quality or composition of milk. A variety of methods are available to assay particular components of milk and milk products. Speed, precision and accuracy are often the key factors that determine the choice of method. Process control samples are usually analyzed by rapid methods, whereas for legal requirements generally requires the use of official reference methods.

10.2 Sophisticated Laboratory Instruments

The sophisticated laboratory instruments often used during analysis of milk and milk products are as follows

- Milko-Tester, Infra Red Milk Analyzer (Milko Scan), Milko-Scan 133-B
- Lactostar automatic milk analyzer
- Lactoscope
- Pro-Milk MK II
- Butyro refractometer
- Rancimat
- Kjeldahl digestion and distillation apparatus
- Flame Photometer
- Atomic Absorption Spectrophotometer

10.2.1 Milko-tester

The Milko-Tester is a product of Danish ingenuity and has been used since 1964. The results with Milko-Tester are comparable to those with the reference method.

10.2.1.1 Principle

Fig. 10.1 Milko tester

The photometric method for determining fat in milk is based on the measurement of light scattering by a diluted sample. A calcium chelating agent, ethylenediaminetetraacetate (EDTA) eliminates the turbidity caused by the casein micelle, so the light scattering is due solely to the fat in the milk. The amount of light scattered is dependent on number and size of fat globules. If the size distribution is uniform the amount of light reaching the photocell will be proportional to the fat content. The results are read from a galvanometer scale graduated in percent fat.

10.2.1.2 Apparatus

There are three models- the Mark II, the Mark III and the automatic. The Mark II model is the least expensive and least automatic. The sample is drawn into the machine by suction where it passes through a 60°C bath and then into a 4-stage homogenizer. The sample is mixed with ethylene di-amine tetra -acetate (EDTA) diluents in a funnel. From the funnel it passes into the flow through cuvette. The beam of light passes through the sample and the amount of light transmitted is detected by a photocell from which a signal is transmitted electrically to the readout meter. Automatic and Mark III models differ from the Mark II by having a digital read out or recorder, which replaces the readout meter, the EDTA diluent from the reservoir passes through a heated container which expels dissolved air, and milk and EDTA are mixed prior to homogenization thereby reducing the size of the sample, increasing the rate of analysis (80 verses 120/hr); minimizing plugging instrument with sour milk, and reducing energy for efficient homogenization. Mark III is semiautomatic faster than Mark II and uses 1.6 ml milk. Milko Tester Automatic (MTA) is fully automatic.

The Milko-Testers are calibrated at the factory against the Rose-Gottlieb method which is the accepted international reference method.

10.2.1.3 Procedure

Calibrate the instrument with milk samples of known fat and SNF content. Take unknown milk sample in the receptacle provided with the instrument. Bring the receptacle below the suction tube of the instrument and run the instrument. Incase of low- cost models sample is homogenized manually using a handle attached with the instrument. Incase of sophisticated instruments every thing is automatic. Record the reading fro Fat and SNF as displayed on the instrument display.

10.2.2 Lactostar automatic milk analyzer

Lactostar a new , versatile, easy to operate microprocessor based , fully automatic milk analyzer for the determination of fat, SNF, protein, lactose and freezing point of milk has been introduced in India recently by FUNKE GERBER, Germany through their sole distributors BENNY IMPEX PRIVATE LIMITED, NEW DELHI.

10.2.2.1 Principle

Fig. 10.2 Lactostar automatic milk analyzer

The Lactostar applies a combined thermo-optical method to analyze the constituents of milk. The device measures both the optical and the thermal properties of the milk. The optical measuring method (Turbidimetric) determines the sum of fat and protein and lactose contents by computational analysis. The freezing point of milk is predicted on the basis of protein content and fat-free dry matter.

10.2.2.2 Procedure

Calibration

For its successful use, it must be calibrated with milk samples of known composition. It needs (i) zero calibration i.e. with distilled water, (ii) A-calibration i.e. with milk of half dilution and (iii) B-calibration i.e. with pure milk. The lactostar has 20 channels and we can calibrate separate channel for each type of different milks. Thus we can have separate calibrated channels for cow milk, buffalo milk, vendors milk, skimmed milk, goat milk, etc.

Take milk sample in the receptacle provided with the instrument. Bring the receptacle below the suction tube of the Lactostar and run the program as stored in the channel of the instrument. Selection of the program depends upon the channel calibrated for a specific type of milk. Run the program and record the results as displayed on the screen or take the print out.

10.2.3 Pro-milk MK II

The Pro-Milk instrument is used for the determination of protein in milk by Dye-binding method. The basis of this method is that certain dyes react stoichiometrically with proteins in acid solutions. After mixing the dye in acid buffer solution with the milk sample, the precipitate is removed and the optical density of the filtrate containing unreacted dye solution is measured in a colorimeter. The first commercially available instrument for dye binding method was produced by Foss Electric Co. Denmark.

10.2.3.1 Principle

The amino groups of arginine, lysine and histidine of milk proteins are involved in binding certain dyes. An aqueous solution dye buffered to about pH 2.0 is added to a milk sample. The protein and the dye that it binds precipitate and are separated from the solution of unbound dye by centrifuging or filtration. The optical density of the filtrate is measured in a colorimeter fitted with a galvanometer type read out. Read out is given in direct percentage protein on a specially designed scale. Dyes used are Amido Black-10 B, Acid Orange-12 and Orange-G.

10.2.3.2 Apparatus

The Pro-Milk MK consists of essentially of a dye container (Dispensing Equipment) with a dispenser pump which discharges a predetermined volume of dye. This dye is mixed with a measured volume of milk in a specially designed mixing chamber (measuring unit). The contents of the chamber are then mixed, reaction being almost instantaneous and the solution filtered under pressure. The filtrate is passed through a colorimeter fitted with galvanometer type read out. Read out gives direct percentage protein on 2.5 – 5.0 scale.

10.2.3.3 Determination

One ml of milk sample is mixed with 20 ml of dye solution in the measuring unit. It is then forced through a filter into the cuvette of the calorimeter. The unreacted dye intensity is determined by

measuring the light transmitted through the cuvette. The reading is taken on the Read out which gives directly the percentage of protein content in the sample. The whole process takes about sixty seconds to complete.

For individual milks, 50 % of the differences between the Kjeldahl values and the Amido-black determinations are due to the variations in the NPN fractions of the sample. The accuracy of the Pro-milk is dramatically improved when true protein is used as reference instead of total protein. Casein reacts at 100 % but non- protein Nitrogenous matter (NPN) practically does not bind dye. Whey proteins bind about 28 % more dye than casein.

10.2.4 Bactoscope/Soma scope

It is an accurate and reliable instrument with which the total bacterial count/ somatic cells in milk can be quantified very quickly and at low costs. The use of automatic settings means that the maximum number of analysis can be reached per hour. Results are given in CFU as per reference method. It also allows the counting of individual bacteria. The Bactoscope does not count directly without incubation. These instruments work on the principle of flow cytometry.In case of somascope a latest flow cytometry technology is used to produce a laminar flow of the somatic cells through the measuring flow cell. This laminar flow ensures that every somatic cell is counted. This results in a linear measurement up to 1*107 cells/ml.

Fig. 10.3 Somascope and Bactoscope

System is total computer based and controlled by the company designed software. Take about 3 ml of sample of milk and heat it to the temperature of 40°± 2C. Put the sample container below the suction tube of the instrument and press the start button. The data is capture by software and saved in the file. It takes about 30 second to analyse one sample .

10.2.5 Rancimat

The Rancimat is a modern PC controlled instrument and allows determination of oxidative stability very comfortably. A rough estimation of the shelf life of a product is possible with unique temperature exploration. In this method the highly volatile organic acids produced by auto oxidation are absorbed in water and used to indicate the induction time.

Fig. 10.4 Rancimat

10.2.5.1 Principle

The Rancimat method developed as an automated version of extremely demanding AOM method (active oxygen method) for determination of induction time of fats and oils. Highly volatile organic acids produced by auto-oxidation are absorbed in water and used to indicate induction time.

Fig. 10.5 Reacton Vessel

10.2.5.2 Procedure

Weigh 3 g sample of completely melted ghee accurately into each of the reaction vessels. Place the vessels in the heating block of the Rancimat apparatus. Then connect the reaction vessels to the measuring vessels via connecting tube. Add 60 ml of deionised water into each of the measuring vessels, containing the electrodes. Now, place the measuring vessels in the Rancimat apparatus. Connect all parts to the apparatus as per the operating instructions, and carry out the test until the induction period of all the samples ends, with a maximum allowable limit of 48 hours.

10.2.6 Butyro refractometer

Refractive index measurements have long been used for the qualitative identification of unknown compounds by comparing the RI of the unknown with literature values of various known substances. The butyro refractometer is widely used to measure the refractive index of fats and oils. An instrument is constructed that measure the critical angle of the sodium D line (589 nm) at 20°C.

Fig. 10.6 Butyro Refractometer

10.2.6.1 Principle

Refractive index and butyro-refractometer (BR) readings, characteristic for the particular liquid or solid, are inter-convertible and are concerned with the degree of bending of light waves passing through a liquid or transparent solid . Generally, it is expressed as the ratio between the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of a definite known wavelength (usually 589.3 nm, the mean of the D-lines of sodium) passes from air into oils and fats.

10.2.6.2 Procedure

Before determining the BR reading of a sample, the temperature of the refractometer was adjusted to 40.0 ± 0.1°C using circulatory water bath and the prisms were cleaned and dried completely. The refractometer was calibrated with the standard provided by the company before taking the reading of the different samples. A drop of the molten fat sample was placed on the lower prism of the refractometer and the prisms were closed and held for 2 minutes. After adjusting the instrument and light to get the most distinct reading possible and bringing the temperature to 40°C, the BR reading of the fat was recorded.

10.2.7 Foss automated Kjeltec

The Danish investigator Kjeldahl worked out in 1883 a method for determining organic nitrogen in his studies on protein changes in grain used in the brewing industry. Basically the sample is heated in sulphuric acid and digested until the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed into ammonium sulphate. Then concentrated sodium hydroxide is added, and the digest heated to drive off the liberated ammonia into a known volume of a standard acid solution. The unreacted acid is determined and the results are transformed, by calculation, into a percentage of protein in the organic sample.

Fig. 10.7 Distillation unit

The FOSS automated Kjeltec™ instruments include systems for sample digestion and distillation. The Kjeltec series is designed to attain the best possible accuracy and precision. It also sets safety standards in Kjeldahl analysis. Also, the Kjeltec™ 2400/2460 is an autosampler system with automatic distillation and titration providing ease of use, speed and accuracy in routine analysis. Operational safety and convenience are enhanced by the unattended operation and features such as automatic reagent addition, tube emptying and a fully integrated safety system. Up to 60 samples can be analysed unattended. The system is truly modular where the automatic distillation/titration unit Kjeltec 2400 can be upgraded to a fully automatic system by the addition of the autosampler system Kjeltec 2460.

Fig. 10.8 Digestion unit

10.2.7.1 Procedure

a) Preparation of test sample

Warm the milk sample (for other milk products, follow the prescribed procedure for sample preparation) to between 38°C to 40°C in the water bath. Gently mix the test sample thoroughly by repeatedly inverting the sample bottle without causing frothing or churning. Cool the sample to room temperature immediately prior to weighing the test portion.

b) Test portion and pre-treatment

To a clean and dry digestion tube, add 12 g of potassium sulfate, 1.0 ml of the copper sulfate solution, approximately 5 g of the prepared test sample of milk and 20 ml of sulfuric acid. Use the sulfuric acid to wash down any copper sulfate solution, potassium sulfate or test portion left on the upper walls of the digestion tube. Gently mix the contents of the tube.

c) Digestion

- Set the digestion block at a low initial temperature so as to control foaming (approximately 180°C). Transfer the tubes to the digestion block and place the exhaust manifold which is itself connected to a water jet pump in the top of the tube. The suction rate of the water jet pump should be just sufficient to remove fumes.
- Digest the sample until white fumes develop. Then increase the temperature of digestion block to between 410°C and 430°C and continue digestion of the sample until the digest is clear.
- After the digest clears (clear with light blue-green colour), continue digestion at between 410°C and 430°C for at least 1 h. During this time the sulfuric acid should be boiling. If visible boiling of the clear liquid is not apparent as bubbles forming at the surface of the hot liquid around the perimeter of the tube, then the temperature of the block may be too low. The total digestion time will be between 1.75 h and 2.5 h.
- At the end of the digestion, the digest shall be clear and free from undigested material. Remove the tube from the block with the exhaust manifold in place.
- Allow the digest to cool to room temperature over a period of approximately 25 min. The cooled digest should be liquid with a few small crystals at the bottom of the tube. Excessive crystallization indicates too little residual sulfuric acid at the end of digestion and may cause a decrease in protein estimation results. To reduce acid loss during digestion, reduce aspiration rate.
- After the digest has cooled to room temperature in approx. 25 min, remove the exhaust manifold and carefully add 85 ml of water to each tube. Swirl to mix while ensuring that any separated out crystals are dissolved. Allow the contents of the tube to cool to room temperature again.

d) Distillation

- Transfer the digestion tube to the distillation unit and place a conical flask containing 50 ml of boric acid solution under the outlet of condenser in such a way that the delivery tube is below the surface of the excess boric acid solution.
- Adjust the distillation unit to dispense 65 ml of sodium hydroxide solution and distill off the ammonia liberated by the addition of sodium hydroxide solution.
- Following the manufacture's instructions, operate the distillation unit in such a way as to steam distil the ammonia liberated by addition of sodium hydroxide solution, collecting the distillate in the boric acid solution. Continue with the distillation process until at least 150 ml of distillate is collected. Remove the conical flask from the distillation unit and completely drain the distillation tip. Rinse the inside and outside of the tip with water,

collecting the rinsing in the conical flask. Always rinse the tip with water between samples. During the distillation, the ammonia solution turns to green.

e) Titration

Titrate the contents of the conical flask with the hydrochloric acid standard volumetric solution using a burette and read out the amount of titrant used. The end point is reached at the first appearance of violet colour in the contents.

f) Blank Test

Carry out a blank test following the procedure described above taking 5 ml of water and about 0.85 g of sucrose instead of test portion.

g) Calculation and expression of results

Calculate the nitrogen content, Wn, by using the following equation:

a. $$W_n = \frac{1.4007\ (V_s - V_b)\ N}{m}$$

Where,

Wn = is the nitrogen content of the sample, expressed as percentage by mass
Vs = is the numerical value of hydrochloric acid standard volumetric solution used in determination in millilitres, expressed to the nearest 0.05 ml
Vb = is the numerical value of the volume of hydrochloric acid standard volumetric solution used in the blank test in millilitres, expressed to the nearest 0.05 ml

N= is the numerical value of the exact normality of the hydrochloric acid standard volumetric solution expressed to four decimal places.
m = is the numerical value of the mass of the test portion in grams, expressed to the nearest 1 mg,

h) Calculation of crude protein content

Calculate the crude protein content Wp using the following equation:

$$Wp = Wn \times 6.38$$

Where,

Wp = is the crude protein content, expressed as a percentage by mass.
Wn = is the nitrogen content of the sample, expressed as a percentage by mass to four decimal places.

6.38 = is the generally accepted multiplication factor to express the nitrogen content as crude protein content

10.2.8 Flame photometer

Early studies during the nineteenth century by J.F.Herschel, D. Alter, and G. Kirchhoff and R. Bunsen laid the foundations for the qualitative differentiations of salts depending on their

emission in a flame. Flame photometer is used for quantitative chemical analysis for the determination of alkali earth metals present in solution. Flame photometer is must where the concentration of the element is very low, say of the order of 1 ppm as ordinary methods such as gravimetric and volumetric will not respond.

Fig. 10.9 Flame photometer

A modern flame photometer consists essentially of an atomizer, a burner, some means of isolating the desired part of the spectrum, a photosensitive detector, sometime an amplifier, and finally a method of measuring the desired emission by a galvanometer. The instruments are used primarily to determine calcium, sodium and potassium from milk and milk products.

10.2.8.1 Principle

Flame analysis is based on the fact that when a metallic salt solution is drawn into a non-luminous flame, it emits light of characteristic wave length. This emitted light, isolated to the characteristic wave band by an optical filter, is allowed to fall on a photocell whose output is measured by a suitable deflection for instance electronic amplifier and a meter or a galvanometer.

10.2.8.2 Preparation of sample

Since a very fine capillary is used to draw the sample into the flame the sample must not contain big solid particles leading to the blockage of the capillary. In case of fluid milk or blood, diluted sample can be used. However, in case of solid products like cheese, khoa, chhana, etc., the sample must be ashed and the ash is then dissolved in dilute acid solution. The concentration of the metal in the diluted sample may be in the range from 5-8 ppm.

10.2.8.3 Preparation of standard solution

Standard solution of the metal or its salt must be prepared in such a way that the concentration of the metal is again 5-10 ppm. For determining a very low concentration of the metal in the unknown sample as well standard must be prepared in some organic solvent or in a mixture of water and organic solvent.

10.2.8.4 Determination of metals in the unknown

For determination of the metal in the sample first of all the instrument must be standardized with a standard solution. Once the instrument is adjusted then the unknown solution is fed to the flame and the reading is recorded in ppm.

10.2.9 Atomic absorption spectrophotometer

Atomic spectroscopy has played a major role in the development of our current database for mineral nutrients and toxicants in milk and milk products. Atomic absorption spectrophotometer (AAS) is widely used and accepted technique capable of determining trace (µg/ml) and ultratrace

(sub- µg/ml) levels of elements or metals in a wide variety of samples, including biological, clinical, environmental, food, and geological samples, with good accuracy and acceptable precision.

Fig. 10.10 Atomic absorption spectrophotometer

10.2.9.1 Principle

Atomic absorption spectrophotometer measures absorption of characteristics radiation by atoms of a particular element to be determined which are thermally atomized either by flame or by graphite furnace. The element which is to be determined is dissolved in a suitable chemical (normally an acid) and this solution is fed into the flame through an aspiration chamber. In case of furnace atomization auto sampler or micro syringe is used for transforming the sample solution into the furnace. A hollow cathode lamp of the element to be determined is used as a source of radiation, which is absorbed by the atoms produced in flame or furnace of that element and absorption is directly proportional to the concentration of the analyte atom. This absorption is compared with the absorption of standard solution of that particular element and actual concentration of element is determined.

10.2.9.2 Sampling and processing of samples

Milk is not totally homogenous because different layers may have different concentrations of minerals. It is, therefore, utmost important that bulk sample be sufficiently homogenized to ensure that the aliquot/sub-sample which is taken for analysis must be representative of the whole. The size of the sample should be proportional to the bulk. Thorough mixing of sub-samples from a large bulk is preferred in representative sampling. When milk sample is taken from a cow, total milk drawn at each milking is mixed and about 100 ml sample is taken in pre-washed stoppered polyethylene bottles. For mineral element analyses, dry ashing or wet digestion can be followed.

(a) Dry Ashing

Generally, 2 to 10 g sample is taken in a silica crucible after preliminary drying of sample. For powdered milk, one gm sample is taken. Then, the sample is placed in the muffle furnace and the temperature is brought to 550°C and held for 4 hr. After cooling, the resulting ash is dissolved in dilute HCl (6M) and then made upto suitable volume. The resulting solution is used in mineral element determination.

10.2.9.3 Analysis of minerals on atomic absorption spectrophotometer

HCl extract or wet digested samples after suitable dilution are used for the estimation of major and trace elements. Major minerals can also be estimated by gravimetric or colorimetric methods apart from AAS, ICP or other techniques used preferably for the analysis of trace minerals.

Lesson 11
PREDICTION OF SHELF LIFE BEHAVIOR OF MILK AND MILK PRODUCTS

11.1 Introduction

Shelf-life is both an important concept and an important property of today's food products.

11.2 What is Shelf Life of Food?

- Defined as the time that a product is acceptable and meets the consumers' expectations regarding food quality
- A guide for the consumer of the period for food can be kept before it starts to deteriorate, provided any stated storage conditions have been followed.
- Describes how long a food will retain its quality during storage.
- Period during which food

    1. Remains safe to eat
    2. Keeps its appearance, texture and flavor
    3. Meets nutritional claims provided on the label, if any

- Thus shelf-life is multifaceted property that is enormously important to food manufacturers and processors as well as consumers. The food safety and desired quality are the two main aspects of an acceptable shelf-life.

11.3 Shelf Life of Food

- Begins from the time the food is prepared or manufactured.
- Indicated by labeling the product with a date mark
- Dependent on many factors
- Types of ingredients, manufacturing process, type of packaging and storage conditions

11.3.1 Declaration about shelf life of food

- Any packaged food with a shelf life of less than two years to be labeled with a date mark.
- Food to be safe up to, and including, the date marked.
- One of the following options must be used:

1. "Use by" date.

- Used for highly perishable foods and present a safety risk if consumed after this date.
- A food must not be sold if it is past its "Use by" date, nor should it be consumed.

2. "Best before" date.

- This is used for foods other than those specified above.
- It is not illegal to sell food that has reached its "Best before" date.
- Specific instructions to be included on the label about storage where these are necessary to ensure that the food will keep for the specified period.
- Storage conditions should be such that they are achievable in the distribution, retail systems and in the home.
- The seller should store the food according to stated storage instructions.

11.4 What is a Shelf Life Study?

Many food products have some variation of open shelf-life dating marked on their containers. These dates help the consumer to decide how long the product may be stored prior to consumption and also help with stock rotation in grocery stores. Therefore, food manufacturer conduct studies to determine the shelf-life of their product. Two methods are used.

1. Direct method

- The most commonly used.
- Involves

    1. Storing the product under preselected conditions for a period of time longer than the expected shelf life
    2. Checking the product at regular intervals to see when it begins to spoil.

2. Indirect method

The approach uses accelerated storage and/or predictive modeling to determine a shelf life.

11.4.1 Direct method for determination of shelf life of food

The most common and direct way of determining shelf-life is to carry out storage trials of the product under controlled conditions that simulate those it is likely to encounter during storage, distribution, retail display and consumer use.
The method involves

- Identification of causes for spoilage of food
- Selection of suitable tests for determining spoilage of food
- Planning of shelf life study
- Running the shelf life study
- Determination of the shelf life
- Monitoring the shelf life

11.4.1.1 Identification of causes for spoilage of food

Three main categories of food spoilage are physical, chemical, and microbiological. List out all the possible ways by which product may deteriorate in quality and/or safety.

11.4.1.2 Selection of suitable tests for determining spoilage of food

An acceptable shelf-life is expected to retain desired sensory, chemical, functional, microbiological and physical characteristics of the product. All tests are not appropriate for all products. For example, one may test milk for numbers of lactic acid bacteria but not fermented milks for these organisms. In general, tests can be divided into four categories viz. sensory, physical, chemical and microbiological.

a) Sensory

Sensory evaluation assesses taste, smell, appearance, and texture of food. It can be used to monitor and record obvious changes that occur over time. Therefore, it is useful when determining the shelf life of a food.

b) Physical

These include tests for measuring product density, viscosity, refractive index conductivity, surface tension, light absorption, redox potential, microscopic examination, etc.

c) Chemical

Chemical tests can detect changes in quality of the product throughout its shelf life. Examples of chemical tests include acidity, free fatty acids soluble nitrogen, peroxide value and headspace gas analysis.

d) Microbiological

These tests can be used to evaluate both food quality and safety. Tests may be done to estimate changes in the number and type of spoilage organism (yeasts, moulds or bacteria) occurring over time.

11.4.1.3 Planning of the shelf life study

The following points to be considered in shelf life study plan:

- What tests need to be carried out?
- How long will the study run for, and how often will the tests be carried out?
- How many samples will be tested each time?
- How many samples will be needed for the whole study period?
- When will the study be run?
- How product, process and packaging are selected?

11.4.1.4 Running the shelf life study

In general, the following are some possible protocols:

- Short shelf-life products
- Chilled foods with shelf-life of up to one week (e.g. ready meals)
- Samples can be taken daily for evaluation
- Medium shelf-life products
- Products with a shelf-life of up to three weeks (e.g. some ambient cakes and pastry)
- Samples can be taken on days 0, 7, 14, 19, 21 and 25.
- Long shelf-life products
- Products with a shelf-life of up to one year e.g. some breakfast cereals and heat-processed shelf-stable foods
- Samples can be taken at monthly intervals or at months 0, 1, 2, 3, 6, 12 and 18.
- he exact frequency will depend on the product and on how much is already known of its storage behavior.

11.4.1.5 Determination of the shelf life

Assigning shelf-lives to products where relevant legal standards are available is relatively straightforward. For instance, in the UK, the Dairy Products (Hygiene) Regulations 1995 specify microbiological criteria for milk-based products, which may be used to fix shelf-lives. Besides

legal standards, voluntary guidelines can also be very useful in assisting companies to define shelf-life endpoints for their products.

- Using all the information which have been observed and recorded, decide how long the product can be kept and still be of an acceptable quality and safety.
- Maximum storage times for quality and safety may not be the same.
- The shelf life of a product should be whichever is shortest.
- Shelf life selected for product should be reasonable, not ideal and should allow safety margin.

11.4.1.6 Monitoring the shelf life

- Samples should be tested for the factors that the shelf life study indicated were the most important for that product, e.g. acidity, loss of flavor, level of spoilage organisms etc.
- Samples should be taken from various points within the distribution and retail system.
- The records made while designing and carrying out the shelf life study will assist in the evaluation of customer complaints, trouble shooting, production and distribution problems and in reviewing the shelf life of the product.
- Continue to monitor the product to ensure it is safe and of good quality throughout its whole shelf life.

11.4.2 Indirect methods for determination of shelf life of food

The food industry has a great need to obtain, in a relatively short time. Consequently, procedures have been developed to predict or estimate shelf-lives quickly. Indirect methods attempt to predict the shelf life of a product without running a full length storage trial; hence, they can be useful for products with long shelf lives. The two most common indirect methods are accelerated shelf life studies and predictive modeling for shelf life.

11.4.2.1 Accelerated shelf life studies

There are number of approaches to accelerated shelf-life testing (ASLT) but all are concerned with how to get reliable deterioration data in a short period, what model to use and how eventually to predict the actual shelf-life of the product.

- In principle, accelerated shelf-life testing is applicable to any deterioration process that has a valid kinetic model.
- That process may be biochemical, chemical, microbiological or physical.
- In practice, most accelerated tests have been done on deterioration processes that are chemical in nature.
- The basic idea is that the rate of a shelf-life limiting chemical reaction is increased at an elevated storage temperature.
- The end of shelf-life is thereby reached much quicker and the data obtained can be extrapolated to provide an estimate of the shelf-life at normal or ambient storage conditions, usually by using the Arrhenius relationship.
- There are two approaches in accelerated shelf-life testing: initial rate approach and kinetic model approach.

I. Initial rate approach

- The simplest technique for accelerating the shelf-life testing is the `initial rate approach.

- It may be applicable to cases where the deterioration process can be monitored by an extremely accurate and sensitive analytical method.
- To predict the actual shelf-life, one needs only to know or to evaluate how the deterioration process behaves as a function of time.
- In chemical reactions that information is provided by the order of reaction .
- Basic to any predictive use of reaction kinetics is that the relationship between the measurable changing reaction parameter and time be linear.
- Labuza (1982) has reported that quality loss follows the following equation

$$dQ/dt = k(Q_A)^n$$

where dQ/dt is the change in the measurable quality factor QA, with time t, k is the rate constant in appropriate units and n is the order of the chemical reaction of the quality factor

- The order of reaction for most quality attributes in food products is either zero, first or second.
- In zero order reactions, the rate of loss of the quality factor is constant or linear and the resulting curve will be linear on a linear plot.
- First order reactions are not linear but are dependent on the amount of the quality factor that remains in the sample at the time.
- Typical first order reactions are rancidity, microbial growth and death, microbial production products, vitamin losses in dried foods, and loss of protein quality.

II. Kinetic model approach

The kinetic model approach is the most common method for accelerated shelf-life testing. The process involves the following steps:

- Selection of desired kinetically active factors for acceleration of deterioration process.
- Running kinetic study of deterioration process at such levels of accelerating factors that rate of deterioration is fast enough.
- Evaluating parameters of kinetic model and extrapolating data to normal storage conditions.
- Use extrapolated data or kinetic model to predict shelf-life at actual storage conditions.

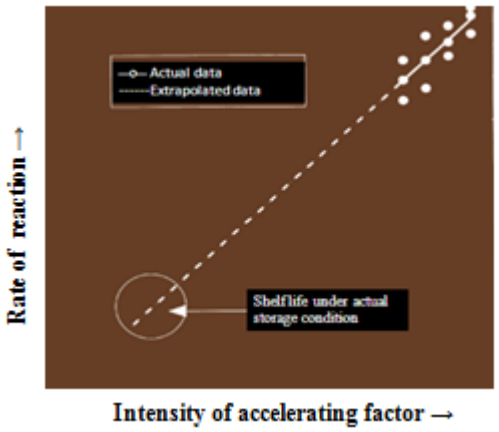


Fig. 11.1 Extrapolation of data in accelerated shelf-life testing

11.4.2.2 Predictive modeling for shelf life

- Predictive models are mathematical equations which use information from a database to predict bacterial growth or chemical change under defined conditions.
- Predictive models can be used to calculate the shelf life of a food.
- Information on the changes that occur in the product when it deteriorates the properties of the product and packaging is required for the calculations.
- Most predictive models are specific to particular types of change.
- Models are useful as a first step in the evaluation of a product's shelf life.
- Information of modeling programme to be verified by challenge testing or a shelf life trial.
- Food safety and technology consultants should be able to assist with specific predictive modeling trials or problems.
- The Arrhenius model that relates the rate of a chemical reaction to the changes in temperature is the best example of such a validated model. This model is represented by the following equation:

$$K = K_0 \exp\left(-\frac{E_a}{RT}\right)$$

where K_0 is a constant, E_a the energy of activation, R the gas constant and T the absolute temperature.

- Each of the chemical deterioration reactions requires a certain amount of energy to get started. This is called activation energy
- The higher the activation energy is for a reaction, the greater the acceleration with increases in temperature.
- A simple way to express this acceleration is to use the Q_{10} concept.

Q_{10} concept

- To simplify the process further, one may get over the need to evaluate K_0 by using a ratio between rates of reaction when temperature is changed by any arbitrary value.
- The most commonly used value is 10°C and therefore the ratio between the rates of reactions is known as Q_{10}. The value of Q_{10} may be calculated using the following equation.

$$Q10 = \frac{\text{Rate of reaction for a temperature of (T+10)}}{\text{Rate of reaction for a temperature of (T)}}$$

- Q10 is an increase in rate of the reaction when temperature is increased by 10°C.
- The rate of reaction being followed is doubled for the 10°C temperature change.
- For example, if a food has a stability of 20 weeks at 20°C, then its stability will be 10 weeks at 30°C. The Q_{10} in this case will be 20/10 that is equal to 2.
- Simplicity of using Q_{10} has made it a very popular method for estimating shelf-life.

1. The popularity of using the Arrhenius model has made it synonymous with accelerated shelf life test.

- Most of the reported accelerated shelf life tests are based on this model.

Lesson 12

PESTICIDES AND ANTIBIOTICS

12.1 Introduction

Environmental contaminants are chemicals that are present in the environment in which the food is grown, harvested, transported, stored, packaged, processed, and consumed. The physical contact of the food with its environment results in its contamination. These include, Pesticides, Vetrinary drugs, Heavy metals, Radionuclides, Polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAH) etc. Possible sources of this contamination are

a) Air
Radionuclides (^{137}Caesium, ^{90}Strontium), polycyclic aromatic hydrocarbons (PAH).

b) Water
Arsenic, mercury.

c) Soil
Cadmium, nitrates, perchlorates. Polychlorinated biphenyls (PCB), pesticides/ insecticides, dioxins, and polybrominated diphenyl ethers (PBDE) are ubiquitous chemicals, which are present in air, water, soil, and the entire biosphere.

d) Packaging materials
Antimony, tin, lead, perfluorooctanoic acid (PFOA), semicarbazide, benzophenone, isopropylthioxanthone (ITX), bisphenol A.

e) Processing/cooking equipment
Copper, or other metal chips, lubricants, cleaning and sanitizing agents.

f) Naturally occurring toxins

Mycotoxins, phytohaemagglutinin, pyrrolizidine alkaloids, grayanotoxin, mushroom toxins, scombrotoxin (histamine), ciguatera, shellfish toxins (see shellfish poisoning), tetrodotoxin, among many others.

12.2 Pesticides

Pesticides are defined as any substance or a mixture of substances used for preventing, destroying, repelling or mitigation of any pest. They are often classified by the type of pest they control e.g. (1) ***herbicides*** to protect the plant from weeds; (2)***fungicides*** to suppress the growth of undesired fungi or molds; and (3) ***insecticides*** to protect the plants from damage caused by insects. In addition to these main groups, there are ***acaricides*** to control mites, ***nematocides*** to control worms or nematodes, ***molluscicides*** to protect the plant from snails and slugs, ***rodenticides*** to control rodents (mice or rats). Pesticides are a key stone components of crop protection. The use of pesticides in food production has provided numerous benefits in terms of increasing production and quality. They are of two types i) Chemical or synthetic organic pesticides and (ii) Biopesticides. Since chemical control of pests is so successful, there has been an

explosive expansion in the development of synthetic organic pesticides. Synthetic organic insecticides including mainly, organochloro (OC), organophosphate (OP) and organocarbamate (OCm) pesticides exhibit a high degree of persistence in the environment as compared to the other classes of pesticides. As a result, consumers are exposed to pesticides posing serious threats to public health by entering into the food chain. Mainly through the crop fields, the pesticides have entered into our food chain and are now omnipresent - in air, water, soil, vegetables, fruits, food grains, animal feeds, meat, milk and milk products.

12.2.1 Classification

Biological effects	Chemical structure	Example
• Insecticides	* Organochlorine(OC)	p,p' DDT
• Fungicides	* Organophosphate(OP)	Malathion
• Herbicides	* Organocarbamate(OCm)	Carbaryl
• Acaricides	* Phenolic	Dintrocresol
• Aphicides	* Dinitroanilines	
• Nematicides	* Triazines	Simazine
• Molluscicides	* Pyrethrines	
• Rodenticides	* Pyrethroids	Cypermethrin

12.2.1.1 Organochlorine(OC)

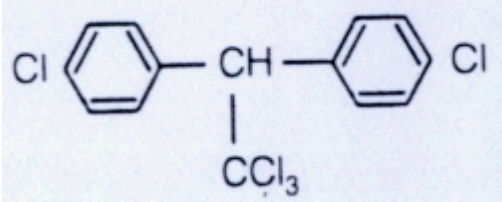


Fig. 12.1(a) p, p' DDT
(Dichlorodiphenyltrichloroethane)

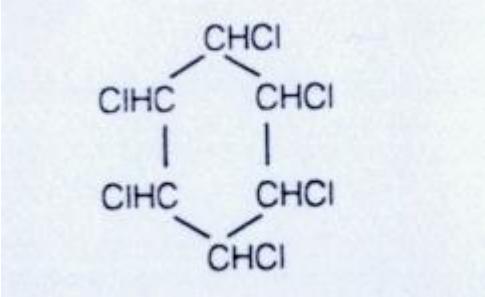


Fig. 12.1(b) BHC
(Benzene Hexachloride)

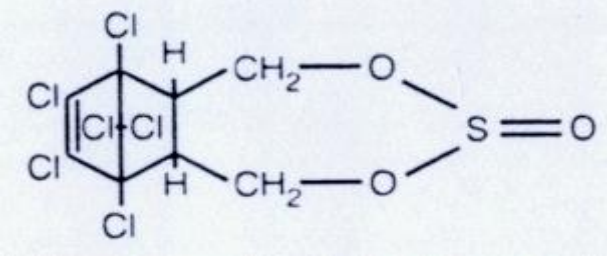


Fig. 12.1(c) Endosulphane

12.2.1.2 Organophosphate (OP)

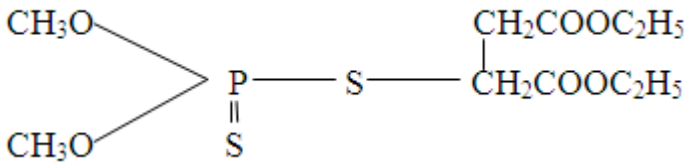


Fig. 12.2 Malathion

12.2.1.3 Organocarbamate(OCm)

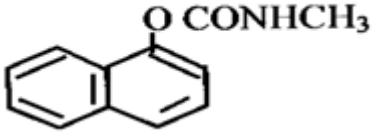
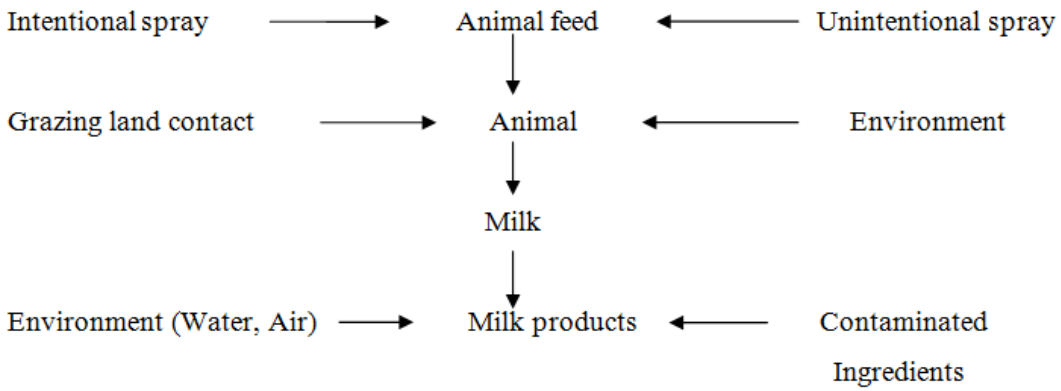


Fig. 12.3 Carbaryl

12.2.2 Source of contamination



12.3 Antibiotics

A drug used to treat infections caused by bacteria and other microorganisms. Originally, an antibiotic was a substance produced by one microorganism that selectively inhibits the growth of another. Synthetic antibiotics, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks. Antibiotics are a boon for the maintenance of the health of the cattle as well as human beings. The judicious use of antibiotics has increased the life expectancy of the human beings as well as cattle. However, indiscriminate use of these life saving products i.e. antibiotics has created problems in the dairy as well as food industry due to there residues coming to milk and meat. The presence of antibiotic residues in milk has a great significance to the dairy industry as these residues may lead to inhibited starter activity, inadequate ripening of cheese and affect flavor and texture of milk products. These residues may also affect consumer's health by causing allergic reactions or by development of resistant microbial strains.

12.3.1 Classification of antibiotics

Antibiotics used in the treatment and management of animals can be chemically grouped in to the following main classes.

A) Aminoglycosides

e.g. Apramycin, gentamicin, lincomycin, streptomycin, neomycin, amikamicin and kanamycin, are having complex but closely related structures. The aminoglycosides are broad-spectrum antibiotics active against both gram-positive and gram-negative organisms but not effective against anaerobes and fungi. They are not well absorbed by alimentary tract or by topical application so they are usually administered intravenously or intramuscularly. This group of antibiotics poses great health hazards, if present in milk and milk products.

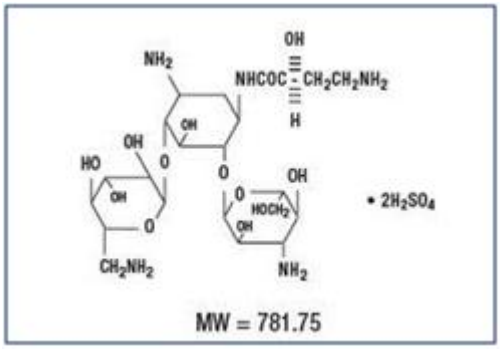


Fig. 12.4 Amikacin sulfate

B) β- Lactam compounds

This group consists of natural penicillin and semi-synthetic penicillin and cephalosporins. Penicillins and cephalosporins interfere in the development of bacterial cell wall and are widely used in the treatment of mastitis. After injection of penicillin-G, the milk of the treated animals' remains contaminated for several days. Cephalosporins are similar to Penicillin in antimicrobial action but less frequently used in veterinary medicine because of its high cost.

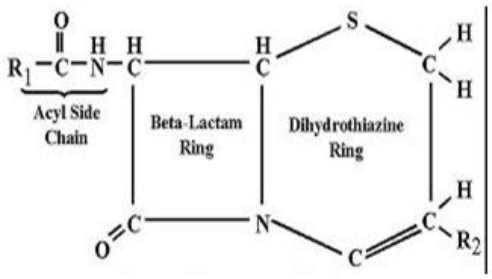


Fig. 12.5 General structure of cephlosporine

C) Macrolides

This group consists of a large lactone ring attached with sugar moieties. It includes erythromycin, spiramycin, tylosin, oleandomycin, clindamycin and roxithrocin. The macrolides are active aginst gram-positive bacteria specially staphylococci which are resistant against penicillin. Many times these compounds are also used as growth promoter. They have better tissue penetration ability and frequently used in veterinary medicine.

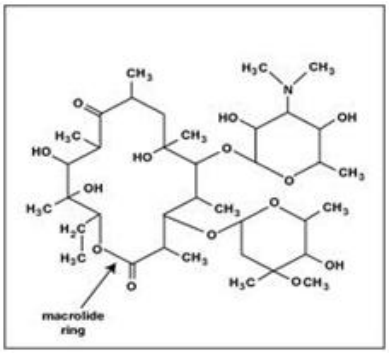


Fig. 12.6 Macrolides

D) Sulfonamides

Sulfonamides interfere with folic acid synthesis thus inhibiting the bacterial growth. Sulfamethaxazole, Sulfadimidine, sulfamethoxypyridazine, sulfaethoxypyridazine incombination with trimethoprin are commonly used in the treatment. In addition sulfaguanidine and sulfaquinoxaline are also used as feed additive. They are broad spectrum and possess good tissue distribution. They have long half-life and have very good chance of its residues in milk of lactating animals.

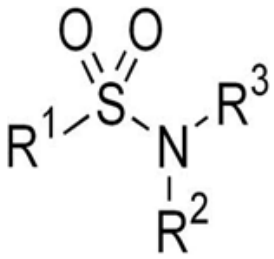


Fig. 12.7 Sulfonamides

E) Tetracyclines

Chlortetracycline, oxytetracycline, rolitetracycline, tetracycline, demethylchlortetracycline and doxycycline are the major antibiotics of this group. The structure consisted of basic naphthalene ring and possesses excellent solubility in aqueous medium. These compounds are active against both gram-positive and negative bacteria. However, their systemic absorption in tissues is slower as compared with penicillin, but are well distributed in tissues and less frequently excreted. Their residual occurrence may be more in milk, bones or calcified organs.

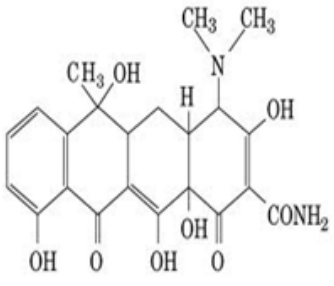


Fig. 12.8 Tetracyclines

12.3.2 Important definitions

For better understanding, it is necessary to understand the terminology by regulatory officials. These terms are designed by number of reports of joint meeting of Food and Agriculture Organization (FAO) and World Health Organization (WHO)

12.3.2.1 Residues

Parent compounds or metabolites of drugs/chemicals, having pharmacological action, if persisted in edible products and are likely to harm human health is called as residue. A residue may also occur if drugs or chemicals or added unintentionally in food products. Residue of a drug is expressed in mg/kg or mg/1000ml (ppm) or µg/kg or µg/1000ml (ppb), ng/kg or ng/1000ml (ppt).

12.3.2.2 Unintentional residues

Unintentional residue is that, which occur in feed and food (milk and meat) as a result of circumstances. Such chemicals are never added to protect the food or feed against infection of bacteria, fungus, or parasite. The unintentional residues also include the residue of a drug or chemical that occurs as environmental contaminants. The unintentional residue cannot be differentiated from residue due to actual use of drug or chemicals.

12.3.2.3 Tolerance levels

It is the maximum permissible residual level, which may be present in tissues or milk of animals. A tolerance level is the maximum allowable level of a drug or chemical in feed or food at a specified time of slaughter, milking, processing, storage, marketing and up to time of consumption by human. There are four types of tolerance

1. Finite Tolerance

It is defined as a measurable amount of drug (no-carcinogen) that is permitted in food. For this purpose the acceptable daily intake (ADI) of human is generally determined by applying the safety factor of 1:100. If the drug or chemical is teratogenic (substances or environmental agents which cause the development of abnormal cell masses during fetal growth) the safety factor of 1:1000 is applied.

2. Negligible Tolerance

The toxicologically insignificant amount of residue, resulting in a daily intake of small fraction of maximum ADI is defined as negligible tolerance. Principle for determination of negligible tolerance is similar to that which is used to calculate finite tolerance except that a factor of at least 1:2000 is used and upper allowable limit is imposed.

3. Zero Tolerance

Zero tolerance is determined on the basis of extent of toxicity of drugs/chemicals. For such chemicals no residue is permitted in feed or food because of extreme toxicity in most of the consumers. Zero tolerance is mostly applicable for carcinogenic drugs.

4. Temporary Tolerance

The temporary tolerance is valid only for the restricted period and subjected to revision of availability of experimental data. Generally it is for new drugs and sometime it is also referred as Interim or Administrative tolerance.

12.3.2.4 Withdrawal time

Time required for a drug concentration to fall below the tolerance level is called as withdrawal time. Sometime it is also referred as "Discard Time" or "Withhold Time". It is expressed in hours, days, weeks or months.

12.3.2.5 Acceptable daily intake (ADI)

The ADI is daily dose of a drug or chemicals residue, which is taken during the entire life-time of a person and appears to be without appreciable risks to health on the basis of all the facts known at that time. ADI value is always subject to revision whenever new information becomes available. It should also be based on the environment and surrounding conditions. It is expressed as mg/kg.

12.3.2.6 Maximum residue limit (MRL)

Maximum concentrations of individual chemicals, or groups of chemicals, especially metabolites, and including pharmaceutical and industrial chemicals, in commodities or tissues to be used as human or animal feeds, and as defined by the food standard codes of a particular country or state; permissible levels vary with local legislation.

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Lesson 13
HEAVY METALS

13.1 Introduction

Metal whose specific gravity is more than 5 g/cm^3. By definition this would account for 60 metals, several of which are biologically essential, and many others lack sufficient information regarding toxicity, including platinum, silver, and gold. These metals belong to a class of ill-defined subset of elements that exhibit metallic properties, which would mainly include the transition metals, some metalloids, lanthanides, and actinides. As far as heavy metals are concerned, soil itself is a good reservoir of such elements. Some of the metals are essential as trace elements (less than 0.001% of the biomass) for humans and other higher animals. However, some metals like **aluminum**, **arsenic**, **cadmium**, **lead**, and **mercury** are toxic even in minute doses. If these elements get their entry into the food chain, they become hazard for the consumers.

13.2 Arsenic

Arsenic appears in three allotropic forms: yellow, black and grey; the stable form is a silver-gray, brittle crystalline solid. The atomic mass of Arsenic is 74.9216. Arsenic compounds are used in making special types of glass, as a wood preservative and, lately, in the semiconductor gallium arsenade, which has the ability to convert electric current to laser light. Arsenic can be found naturally on earth in small concentrations.

13.2.1 Sources of arsenic contamination

These include air, antibiotics given to commercial livestock, certain marine plants, chemical processing, coal- fired power plants, defoliants, drinking water, drying agents for cotton, herbicides and pesticides, insecticides, meats (from commercially raised poultry and cattle), metal ore smelting, seafood (fish, mussels, oysters), specialty glass, and wood preservatives.

13.2.2 Health hazards

Exposure to inorganic arsenic can cause various health effects, such as:

a) Irritation of the stomach and intestines
b) Decreased production of red and white blood cells,
c) Skin changes and lung irritation.
d) More risk of cancer (skin cancer, lung cancer, liver cancer and lymphatic cancer) on prolonged and significant uptake of inorganic arsenic.
e) Infertility and miscarriages in women on very high exposure to inorganic arsenic.
f) Damage to DNA at high doses of inorganic arsenic. A lethal dose of arsenic oxide is generally regarded as 100 mg.

13.3 Lead

Lead is a bluish-white lustrous metal having molecular mass of 207.2. It is very soft, highly malleable, ductile, and a relatively poor conductor of electricity. Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements. Native lead is rare

in nature. Currently lead is usually found in ore with zinc, silver and copper and it is extracted together with these metals.

13.3.1 Sources of lead pollution/ contamination

The main sources of lead pollution in the environment are

a) Industrial production processes and their emissions, road traffic with leaded petroleum (now problem is almost nil because lead free petrol is available in the market), the smoke and dust emission of coal and gas.
b) Ammunition (shot and bullets), bathtubs (cast iron, porcelain, steel), batteries, canned foods, ceramics, chemical fertilizers, cosmetics, dolomite, dust, foods grown around industrial areas, gasoline, hair dyes and rinses, leaded glass, newsprint and colored advertisements, paints, pesticides, pottery, rubber toys, soft coal, soil, solder, tap water, tobacco smoke, and vinyl mini-blinds.
c) Vegetables with larger surface areas (spinach, cabbage) may contain higher levels of lead when cultivated near the lead emission source.

13.3.2 Health hazzards

Lead ingestion leads to the following health hazards

a) Inhibition of the synthesis of red blood cells, which in turn compromises oxygen transport.
b) Effect on bone marrow, liver, nervous system, reproductive tissues, and kidney, due to increase in the binding capacity of blood proteins.
c) Injuries to mental development with reduction in intelligence, growth, and cognitive function especially by organic lead compounds.

13.4 Mercury

It is a heavy, silvery-white liquid metal with atomic mass of 200.59. Mercury is sometimes called quicksilver. The most important mercury salts are mercuric chloride $HgCl_2$ (corrosive sublimate - a violent poison), mercury fulminate $Hg(ONC)_2$, a detonator used in explosives) and mercuric sulphide (HgS, vermillion, a high-grade paint pigment). Mercury occurs uncombined in nature to a limited extent. It rarely occurs free in nature and is found mainly in cinnabar ore (HgS) in Spain, Russia, Italy, China and Slovenia. Mercury is not naturally found in foodstuffs, but it may turn up in food as it can be spread within food chains by smaller organisms that are consumed by humans, for instance through fish. Mercury concentrations in fish usually greatly exceed the concentrations in the water they live in. Cattle breeding products can also contain eminent quantities of mercury. Mercury is not commonly found in plant products, but it can enter human bodies through vegetables and other crops, when sprays that contain mercury are applied in agriculture. It also gets concentrated in shellfish, crustaceans, and fish, and passes on in the food chain in its highly toxic form, methylated mercury. Methyl mercury compounds are the most toxic of heavy metals.

13.4.1 Sources of contamination/pollution

Mercury enters the environment as a result of

a) Normal breakdown of minerals in rocks and soil through exposure to wind and water.
b) Catalysts, thermometers, and pigments, batteries, cosmetics, dental amalgams, diuretics

(mercurial), electrical devices and relays, explosives, foods (grains), fungicides, fluorescent lights, freshwater fish (especially large bass, pike, and trout), insecticides, mining, paints, pesticides, petroleum products, saltwater fish (especially large halibut, shrimp, snapper, and swordfish), shellfish, and tap water.
c) Emissions from chemical plants, power stations, often as effluents and sludge.

13.4.2 Health hazards

Mercury poisoning caused by food intake is derived from organomercury compounds, e.g., dimethyl mercury (CH_3 Hg CH_3), methyl mercury salts (CH_3 Hg; X=chloride or phosphate), and phenyl mercury salts (C_6H_5 Hg X; X=chloride or acetate). These highly toxic compounds are lipid soluble, readily absorbed and accumulate in erythrocytes and the central nervous system.

a) Ingestion of organic mercury results in distribution to the liver, kidneys, and brain.
b) Tiredness, loss of appetite, weight loss, muscular weakness, perhaps paralysis, and eventually kidney failure.
c) Damage to the central nervous system and the immune system, and has been shown to produce teratogenic effects.
d) Allergic reactions, resulting in skin rashes, tiredness and headaches. Negative reproductive effects, such as sperm damage, birth defects and miscarriages

13.5 Cadmium

Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife. Atomic mass of Cadmium is 112.4. Cadmium is a naturally occurring minor element, one of the metallic components in the earth's crust and oceans, and present everywhere in our environment. It is soluble in acids but not in alkalis. It is similar in many respects to zinc but it forms more complex compounds. Cadmium is released into rivers through weathering of rocks and some cadmium is released into air through forest fires and volcanoes. The rest of the cadmium is released through human activities, such as manufacturing.

13.5.1 Sources of contamination/pollution

Contributions to residues include

a) Industrial processes such as metal refining, coal and oil industry, and electroplating plants.
b) Air pollution, art supplies, bone meal, cigarette smoke, food (coffee, fruits, grains, and vegetables grown in cadmium-laden soil, meats (kidneys, liver, poultry, or refined foods), freshwater fish, fungicides, highway dusts, incinerators, mining, nickel-cadmium batteries, oxide dusts, paints, phosphate fertilizers, power plants, seafood (crab, flounder, mussels, oysters, scallops), sewage sludge, softened water, smelting plants, tobacco and tobacco smoke, and welding fumes.

13.5.2 Health hazards

Cadmium is first transported to the liver through the blood and affects the health :

a) Binding to proteins to form complexes that are transported to the kidneys. Cadmium accumulates in kidneys. A level of 0.2–0.3 mg Cd/g kidney cortex causes damage of the tubuli and thereby causes the damage to filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further kidney damage.

b) Other health effects that can be caused by cadmium are:- **(i)** Diarrhoea, stomach pains and severe vomiting **(ii)** Bone fracture **(iii)** Reproductive failure and possibly even infertility **(iv)** Damage to the central nervous system **(v)** Damage to the immune system **(vi)** Psychological disorders **(vii)** Possibly DNA damage or cancer development.

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Lesson 14

METHODS OF ESTIMATION OF PESTICIDES, ANTIBIOTICS AND HEAVY METALS

14.1 Introduction

Residues of antibiotics and pesticides are detrimental to health of the consumers as well as affect the quality of the dairy products. Presence of these contaminants in the milk and milk products is through the indiscriminate use of antibiotics in the health management of cattle and excessive use of pesticides in the fields. These contaminants not only affect the health and quality but also the export/ import potential of the dairy products. Similarly, heavy metals also have health hazards. In the present era of globalization the quality is the top most considered factor in running the business. Since, these contaminants are present in very small amounts, which are difficult to detect by the routine or old methods so sensitive methods are to be used. Thesis sensitive methods help in ensuring the presence or absence of these contaminants in the food stuffs. Some of the methods are as follows:

14.2 Methods for Pesticides

Isolation of organochloro pesticide residues (OCPR) in milk and milk products essentially is based upon adsorption clean-up (IDF standard 75 C: 27 method F, 1991). This involves mixing of test portion in presence of water with florisil until a homogenous powder is obtained, transfer of this mixture to florisil column, selective elution of pesticides and concentration of the eluate followed by HPLC analysis. The sample isolates are analysed by standardised HPLC conditions on octadecylsilyl (ODS) column, with solvent system methanol: water (80:20) at flow rate of 1.0 ml/min and at wavelength of 254 nm. Gas liquid chromatography (GLC) with electron capture detector (ECD) can also be used for the analysis of OCPR. Isolation of multiresidue of pesticides in milk is based upon solid phase extraction (SPE) over C18 cartridges. This involves blending of milk sample with acetonitrile followed by collection of supernatant over anhydrous sodium sulphate. The supernatant is concentrated to small volume, passed through SPE cartridges, eluant evaporated and again dissolved in known volume of acetonitrile followed by High pressure liquid chromatography (HPLC) analysis using binary gradient programming of acetonitrile and water solvent system. The sample isolates of multiresidue of pesticides are analysed by standardised HPLC conditions on ODS column, with solvent system acetonitrile: water (75:25) at flow rate of 0.5 ml/min and at wavelength of 200 nm. GLC with nitrogen phosphorus detector (NPD) can also be used for the analysis of organophosphates and carbamates.

Fig. 14.1 Florisil

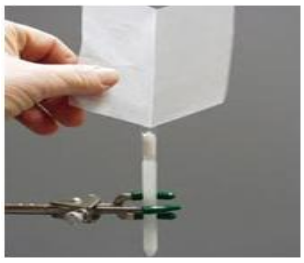

Fig. 14.2 Florisil column filling

14.3 Methods for Antibiotics

14.3.1 Routine test methods for detection of antibiotic residues in milk

Rapid detection of antibiotic residues in milk is of immense importance to the dairy industry. Various rapid antibiotic detection methods have been commercialized in last two decades. Currently, seven types of detection methods are commonly used for detection of antibiotic residues in milk i.e. microbial growth inhibitor assay, microbial receptor assay, enzyme-colorimetric assay, receptor binding assay, spectrophotometric assay, chromatographic methods and immunoassay. These methods are qualitative, quantitative or semi-quantitative. However, they have one or more limitations in terms of precision, accuracy, sensitivity, cost and infrastructural requirement. Currently, microbial inhibitor & immuno-receptor tests have gained most popularity in the dairy industry at international level.

14.3.1.1 Reference method

The EU reference method for the determination of antibiotic residues in raw milk and in heat-treated milk is the International Dairy Federation microbial inhibition test. The IDF microbial inhibitor test uses *B. stearothermophilus var. calidolactis*, ATCC 10149 as the test organism due to its relatively high sensitivity to inhibitory substances. The IDF test procedure for detection of antibiotic residues in milk has been chosen as representative of similar procedures which in principle use *B. stearothermophilus* as the test organism. However, the IDF method is quite complex and lengthy to carry out as it involves the continual growth of large quantities of *B. stearothermophilus* spores. As the test involves a color change, which is dependent on the growth of *B. stearothermophilus*, if the organism fails to grow then a false negative result may occur. Therefore, due to the aforementioned technical difficulties in carrying out the reference method, microbial inhibitor test kit assays based on the IDF method, using B. stearothermophilus are the routine methods used for the determination of antibiotic residues in milk.

14.3.1.2 Microbial Inhibitor test

The 'traditional' tests for antibiotics in milk, known as 'microbial inhibitor' tests, involve incubating a susceptible organism in the presence of the milk sample. In the absence of an antibiotic, the organism grows and can be detected visually either by opacity of the agar growth medium or by a color change resulting from acid production. In the presence of an antibiotic, or any other inhibitor, the organism fails to grow and a zone of inhibition or lack of a color change is observed. Such tests are exceptionally sensitive to ß-lactam antibiotics. They are generally reliable and cost-effective but require incubation for several hours before the result can be visualized.

14.3.1.3 Commercially available microbial inhibitor test

Based on the microbial inhibitor test principle several commercially available kits are popularly used. The Delvotest (Gist-brocades BV, The Netherlands) is the best known microbial inhibitor test. The first version developed, in the 1970s, was the Delvotest P, designed to detect ß-lactams. The target organism, *B. stearothermophilus*, is encapsulated in an agar medium containing a pH indicator, a nutrient tablet and the substantial excretion of these residues into milk sample both being dispensed onto the agar surface. The 'ampoule version' is designed for individual tests or small-scale testing whilst a micro-tire plate version is designed for mass testing where 96 tests can be undertaken simultaneously. A negative result is indicated by a color change from purple to yellow, due to acid development during incubation at 64°C for 2½ hours. The Delvotest P has been used throughout the world and has sensitivity to penicillin G of 0.005 IU/ml. A more recent

development, the Delvotest SP, is capable of detecting a wider spectrum of substances, notably sulphonamides, but also has increased sensitivity to tylosin, erythromycin, neomycin, gentamicin, trimethoprim and other antimicrobials. The Delvotest SP appears identical to the Delvotest P, the only difference being the need to incubate the Delvotest SP for 2¾ hours. The Delvotest SP is sold throughout the world and, universally, has sensitivity to penicillin G of 0.003-0.004 IU/ml.

The Delvotest was introduced into the UK in 1994 for testing individual animals as well as bulk tank milk and is identical to the ampoule version of the Delvotest P, differing only in its packaging. Although the Delvotest is by far the most widely used microbial inhibitor test, Charm Sciences Inc. (USA) has manufactured three similar tests. The Charm AIM-96 test is a micro-tire plate test, similar to the Delvo test and capable of detecting ß-lactams, sulphonamides, tetracycline, macrolides and amino glycosides in 96 samples simultaneously. Unlike the Delvotest, however, it employs a liquid medium instead of agar. The inoculated micro-tire plate is incubated on a heating block, programmed to provide a time-temperature profile suited to the batch of *B. stearothermophilus* spores being used; the incubation period is typically 3-4 hours, at the end of which a blue-yellow color change indicates that a sample is negative. The Charm Farm test is a 'test-tube' version of the AIM-96 test, designed for on-farm use and employs the same microbial inhibitor principle with a color change.

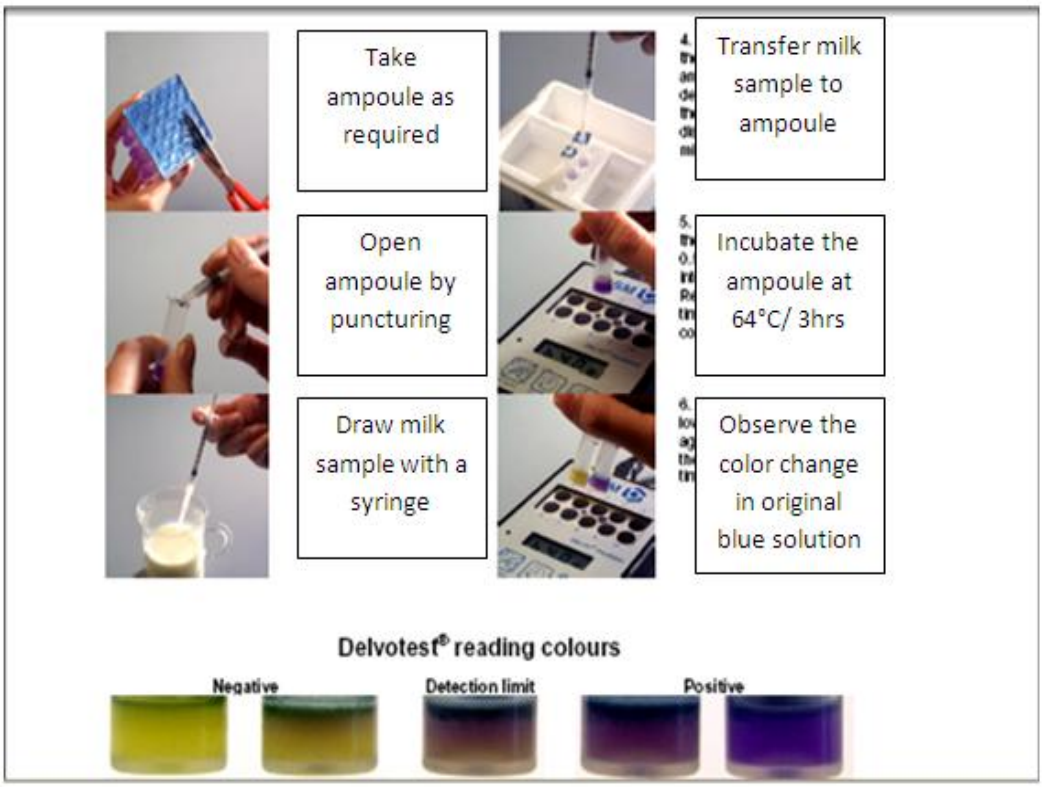

Fig. 14.3 Microbial inhibitor test

14.3.2 Rapid test kits for the detection of antibiotics in milk

Microbial inhibition tests are lengthy and test for a broad spectrum of antibiotics, whereas rapid test kits generally detect a specific family of antibiotics. To avoid delays at milk intake points, rapid antibiotic screening tests are often used on raw milk prior to completion of the Delvo® SP test.

14.3.2.1 Immuno-receptor test

The desire for a more rapid and reliable result has promoted the development of tests that employ the 'immune receptor' test principle, which is a variation of the well-established enzyme-linked immunosorbent assay (ELISA). Essentially, a specific target antibiotic group is captured by immobilized antibodies, or by a broader-spectrum receptor such as a bacterial cell. Most tests involve a competitive principle in which antibiotic in the sample competes with an internal antibiotic standard for the immune receptor. The antibody-antibiotic complex is then usually linked to an enzyme that catalyses a color or fluorescence reaction and a comparison of the intensity of the 'test' reaction with that of a 'control' determines whether the sample is positive or negative. Because of their competitive principle, a low intensity usually means 'positive' whilst a high intensity is regarded as 'negative'. Immune receptor tests can be made quantitative but are generally used to provide a 'pass/fail' result. They are generally more expensive than microbial inhibitor tests but only detect substances that react immunologic ally with the immobilized receptor and they provide a result in less than 10 minutes.

14.3.2.2 Commercially available immuno-receptor test

The commercially available immune receptor tests employ several variations of capture mechanism and color reaction but most possess the common features of an immunological reaction coupled with a change in color (or fluorescence). There are, however, two exceptions. The Penzym test (UCB Byproducts, Belgium) employs the inhibition of an enzyme reaction (DD-carboxypeptidase's activity), instead of an immune reaction, to detect the presence of a ß-lactam and it visualizes this by a color change. The test produces pink color when a sample contains no antibiotics while a yellow color is interpreted as positive. Conversely, the Charm II assay (Charm Sciences Inc., USA) employs an immune reaction to bind the antibiotic to a microbial receptor but detects this complex using a low-level 3H or 14C radio-label, instead of an enzyme reaction. The Charm II assay (Charm Sciences Inc., USA) is not a single test but a family of separate tests for specific groups of antibiotics, notably ß-lactams, sulphonamides, tetracycline, novobiocin, amino glycosides and macrolides, as well as various other substances such as chloramphenicol. The Charm II assay is an immune receptor test but is suitable for large laboratories only, requiring a range of laboratory equipment, including a centrifuge and sample mixers to prepare samples as well as a scintillation counter to detect the radio-label. Calibration curves need to be prepared for each group of antibiotics and a 'negative control' sample must be tested each day. The charm II β-lactam test uses bacteria with specific receptor sites that bind all β-lactam drugs. The bacteria are added to a milk sample along with an exact amount of [14C] labeled penicillin G. Any β-lactam already in milk competes for the binding sites with the labeled penicillin G. The amount of [14C]-penicillin G that binds to the receptor sites is measured compared to a previously determined control point or to a standard curve. The greater the amount of [14C]-penicillin G measured, the lower the β-lactam concentration in the sample. The Charm MRL test (Charm Sciences, USA) is very similar to the Beta STAR test and detects penicillin and cephalosporin in 8 minutes. The test strip is placed in a heating block, the milk sample is added to an absorbent pad at one end and the test is incubated. Two lines appear on the dipstick, a sample being considered positive if the 'test' line is lighter than the 'control' line. The results can be read visually or using an image reader.

14.4 Methods for Heavy Metals

14.4.1 Atomic absorption spectrometery

For routine analysis of heavy metals the method of choice is the atomic absorption spectrometry (AAS) which makes use of aqueous digest of the sample. The main approaches in this technique

are either the wet acidic digestion of milk and/or milk products or the dry ashing to yield the final inorganic extract, suitable for flameless determination in the AAS apparatus. Most preparation procedures lead to undesirable high backgrounds levels from chemicals and/or the digestive apparatus and may therefore lead to false results. For the determination of arsenic, selenium, and mercury, special AAS techniques with the thermal decomposition of arsenic gas, selenium hydride or the cold absorption of mercury vapors are frequently used and yield reliable results. Neutron activation analysis (NAA) is based on element specific γ radiation of irradiated elements, but is not suitable for routine as not all elements of interest can be analysed due to nuclear safety regulation. Other procedures are spectrophotometric methods, voltametric and isotop dilution mass spectrometry.

14.4.2 Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

ICP-AES, also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element.

14.4.3 Inductively coupled plasma-mass spectrometry (ICP-MS)

ICPMS is a relatively new technique for the determination of trace elements in solution. It offers better sensitivity than graphite furnace AA with the multi-element speed. It is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentrations below one part in 1012. It is based on coupling together a high-temperature ICP (inductively coupled plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer. In a typical application, metals are placed in solution by acid digestion. The solution is sprayed into flowing argon and passed into a torch which is inductively heated to approximately 10,000^{o}C. At this temperature, the gas and almost everything in it is atomized and ionized, forming a plasma which provides a rich source of both excited and ionized atoms. In ICPMS, positive ions in the plasma are focused down a quadrapole mass spectrometer. By acquiring the mass spectrum of the plasma, data can be obtained for almost the entire periodic table in just minutes with detection limits vary from metal to metal and ranges from 0.1 – 100 ppb depending upon the type of elements. This method requires a very small amount of sample about 10 mg. This technique has the following advantages:

i. Detection limits for most elements equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
ii. Higher throughput than GFAAS
iii. The ability to handle both simple and complex matrices with a minimum of matrix interferences due to the high-temperature of the ICP source
iv. Superior detection capability to ICP-AES with the same sample throughput
v. The ability to obtain isotopic information.

Lesson 15
MILK CONTACT SURFACES

15.1 Introduction

Most of the foodstuffs sold are packaged. The packaging shall protect the food but shall not contaminate the packed content. Migration refers to the transfer of a substance from the packaging to the foodstuff inside and vice versa. Plastics are most commonly used as packaging material. They are made from monomers and other starting substances which are chemically reacted to a macromolecular structure, the polymer. The polymer as such is an inert high molecular weight structure. As substances with a molecular weight above 1000 Dalton usually cannot be absorbed in the body, the potential health risk from the polymer itself is minimal. Potential health risk may occur from non- or incompletely reacted monomers or other starting substances or from low molecular weight additives which are transferred into food via migration from the plastic food contact material.

15.1.1 Definition

- All materials and articles intended to come into contact with foodstuffs, including packaging materials, cutlery, dishes, processing machines, containers etc.
- It also includes materials and articles which are in contact with water intended for human consumption but it does not cover fixed public or private water supply equipment.
- Food contact materials can be constructed from a variety of materials like plastics, rubber, paper, coatings, metal etc. In many cases even their combinations are used.
- Different types of additives such as antioxidants, stabilizers, lubricants, anti-static, anti-blocking agents, etc. are added to improve performance of polymeric packaging materials.
- Direct contact between the food and its packaging may results in the migration of packaging components into food.
- Recently the packaging has been found to represent a source of contamination itself through the migration of substances from the packaging into food

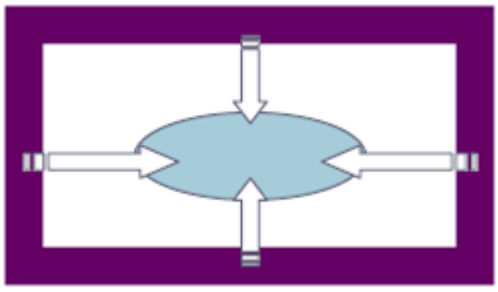

Fig. 15.1. Migration of molecules from the package to the product

15.1.2 Safety

- The food contact materials shall be safe.
- They shall not transfer their components into the food in quantities that could endanger human health, change the composition of the food in an unacceptable way or deteriorate the taste and odor of foodstuffs.

- During the contact of the food contact materials with the food, molecules may migrate from the food contact material to the food. Because of this, in many countries regulations are made to ensure food safety.
- The transfer of constituents from food contact materials into food is called migration. To ensure the protection of the health of the consumer and to avoid any contamination of the foodstuff two types of migration limits have been established for plastic materials by EU legislation

Specific Migration Limit (SML)

- SML means the maximum permitted amount of a given substance released from a material or article into food or food simulants.
- SMLs are a risk management tool derived from toxicological data, such as tolerable daily intakes (TDIs), or from a limited toxicological assessment ensuring safety only for a low migration
- Plastic materials and articles shall not transfer their constituents to foods in quantities exceeding the set SML.
- The SML are expressed in terms of mg of substance per kg of food or food simulants.
- For substances for which no specific migration limit or other restrictions are provided, a generic specific migration limit of 60 mg per kg shall apply.

Overall Migration Limit (OML)

- OML means the maximum permitted amount of non-volatile substances released from a material or article into food simulants.
- The OML may be seen as a restriction of food contamination by the sum of the substances migrating from food contact materials.
- Plastic materials and articles, intended to be brought into contact with food, shall not transfer their constituents to food simulants in quantities exceeding 60 milligrams of total of constituents released per kg of food or food simulant.
- Therefore, plastic materials and articles shall not transfer their constituents to food simulants in quantities exceeding the stipulated limits.

15.1.3 Methods for testing of packaging materials

- Under mandate of European Commission the European Committee for Standardization has prepared standard test methods required for testing of compliance with the requirements and restrictions in the plastics Directives. Overall migration test methods are published in 1 to 12 parts of EU Commission Regulation on plastic materials and articles intended to come into contact with food.
- Specific migration test methods of seven plastic monomers are published in eighth parts of the EU Commission Regulation.
- Methods of analysis for 35 monomers are developed in a European research project and published in European Commission Regulation.
- Test methods to be used for checking paper and board are prepared and published for

1. preparation of cold and hot water extracts
2. determination of water soluble matter, formaldehyde, polychlorinated biphenyls and metals (cadmium, lead, chromium, mercury)
3. determination of fastness of coloring agents and fluorescent whitening agents
4. transfer of anti-microbial constituents

Testing is carried out using food simulant

Rules for selecting simulant and test conditions are given in the relevant EC directives.

15.2 Milk Contact Materials

- Milk is a fluid, therefore, it's handling while collection, transport, processing, product manufacturing, storage and marketing requires use of containers, equipments
- Some common articles and material likely to come in contact with milk from production to consumption chain; at various stages like milking, storage, transport, processing, packaging, distribution and utilization are:

    - Active or intelligent materials
    - Adhesives
    - Ceramics
    - Coatings and lacquers
    - Cork
    - Glass
    - Ion-exchange resins
    - Metals and alloys
    - Paper and board
    - Plastics
    - Printing inks
    - Regenerated cellulose
    - Rubbers
    - Silicones
    - Textiles
    - Varnishes and coatings
    - Waxes
    - Wood

The brief account of different milk contact surfaces is presented below.

15.3 Metals

Milk has to find its place in any container after leaving the udder of the milk animal. These containers are usually made up of metals. As soon as milk occupies its place in the metal container that we use, it starts reacting with the metal. If it is of good quality, the milk will remain good and if it is not then, it is difficult to maintain the quality of milk produced both from the stand point of clean milk production and good flavors.

With respect to flavor, keeping quality and safety of the product; some of the above important considerations are important to discuss as 'effect of milk on metals'. The metallurgy of the dairy plant is very different from the other industries. A slight change in the composition of the metal greatly affects the working quality of the equipments and wholesomeness of milk and milk products. The corrosive properties of milk are due not only to various mineral salts in solution but also due to its protein content.

The solubility of metals in milk and other dairy products is of interest both from the standpoint of durability of the equipment and of the effect of the dissolved metals on the flavor, keeping quality, and healthfulness of the product. Aeration favors corrosion and corrosion is greatest at the milk-air junction. Acid milks are more corrosive.

15.3.1 Aluminium

One of the common single metal, second best to stainless steel is used in dairy equipments. Aluminium is not appreciably attacked by milk. It is however strongly corroded by sodium hydroxide and by alkaline solutions. The pure metal is rather soft for use and usually alloys are now employed, especially those incorporating silicon. In cleaning aluminium plant and utensils, care must be taken to incorporate a fair proportion of sodium metasilicate in detergent. Souring of milk and whey also attacks aluminium and cause pitting. Phosphoric acid used for removing milk-stone on pasteurizing plant also attaches aluminium. Aluminium is non-toxic and no flavor is imparted to milk until 9 ppm is reached. The alloys which appear to be most suitable for use in the dairy industry are those of aluminium with silicon, manganese and magnesium. Now-a-days it is used in storage tanks, rail tankers, butter churns and cans.

15.3.2 Stainless steel (SS)

Mostly all the modern dairies extensively use S.S. because of its high resistance to corrosion, easy to clean, bright, good heat conductor, gives nice appearance and imparts no taints and off flavor to milk and milk products. The S.S. consists of 18 parts of chromium and 8 parts of nickel. Addition of 3% molybdenum further improves the resistance of this 18:8 to corrosive influences.

15.4 The Effects of Metals on Milk and Milk Products

The passage into milk of any particular metal may have two undesirable consequences in addition to corrosion. It may impart a bitter, metallic flavor and initiate the cycle of changes leading to an oxidized taints. Instead, of one, if two metals enter milk to the same extent, the effect may be doubled or will be very different considering the sufficient emphasis upon the flavor and keeping quality of milk-products, the effect of metal is of great importance. The classic work done by various workers has shown conclusively that some of the common metals do impart objectionable flavors to milk under certain conditions.

There is considerable weight of evidence to show that extremely small traces of heavy metals, nickel, manganese, chromium in general and copper in particular exercise a powerful catalytic effect to develop an oxidative deteriorations. These metals find their way into milk through the worn coolers in which the tinned surface is no longer intact. Milk is thus in contact with tin and copper at the same time and as a consequence copper dissolves under the influence of protein also favor solutions of copper through the formation of copper-protein complex, which become absorbed by the fat globules and in this manner, the metal is brought into contact with the fat. Iron, cobalt, nickel, chromium and manganese are also effective, but to a much lesser degree than copper. Tin and aluminum have little effect, copper is a normal constituents, being present to the extent of 0.12 ppm but if the concentration rises to 1.5 ppm, an oily taint may result.

Cream, butter and whole milk powder are similarly sensitive to the catalytic effect of copper. It has been found that a number of factors affect the degree of action of the metal. If the milk is warm, metal surface is not clean and highly polished (un-cleaned and dull surface), high acidity of milk together with sugar the effect is likely to be greater. The effect of nickel, manganese, chromium and iron are not as severe as those of copper but hastened by acidity. In addition to its effect on flavor, fat oxidation is known to cause the losses of vitamin A and vitamin C (ascorbic acid). In case of whole milk powder, concentration of copper as low as 4 ppm has a strong catalytic effect on the oxidation reactions and again this is assisted by presence of moisture.

Fishiness and tallowy flavor defects in butter due to high concentration of acid is further

aggravated by metallic contamination. The effect of metallic contamination was studied by adding various quantities of metallic lactates during the salting of butter. Tallowy taint developed so quickly that no first fishiness could be detected.
The possibility of metallic contamination also arises from the metal foil used for capping bottle milk. Aluminum foil is generally used but when lacquered zinc foil is used, normally the foil is in contact with cream layer the tinned lead allows quantities of lead to pass into milk to a degree which would render it unsafe for human consumption.

15.4.1 Pasteurized milk

Milk is brought by the vendors in aluminium containers to the doorsteps of the consumers, where the required quantity is measured and given out in the customer's container. In the packed form, milk is sold in returnable glass bottles sealed with aluminium foil cap clear glass bottles of 500 ml capacity. However, due to the handling problems of glass and other related issues, an alternate packaging system was evolved in the early 80's, and thus plastic pouches replaced glass bottles. Plastic pouches are generally made of low-density polyethylene (LDPE film). Co-extruded LDPE-LLDPE film is also used because of its advantage of eliminating pin-hole problems. The films are of 65-70 μm thick.

Another technological breakthrough in processing and packaging of milk is the Aseptic Packaging, commonly known as the Tetra pack milk. In this packaging system, both the package and the product are sterilized separately and the packaging operation is carried out under aseptic (sterile) conditions. This system offers a long storage life of about 3 months, without the need for refrigeration or added preservatives. A tetrapak carton is formed from a composite material, which has 5 to 7 layers including paperboard, aluminium foil and polyethylene.

15.4.2 Flavored milk

For flavored milk drinks the package should be leak and tamper proof, should have sufficient wet strength and should not pass on any odor or taint to the product packed inside. The plastic based material used for sachets is octane LLDPE (O-LLDPE). OLLDPE when blended with 50% LDPE provides excellent puncture resistance, excellent seal strength and hot tack. In India, flavored milk drinks are available in sterilisable crown cork glass bottles, glass bottles with aluminium foil lid or snap-on plastic lid, plastic sachets and aseptic packs (Tetra bricks). Recently 200ml, translucent bottles of HDPE with an aluminium foil cap have also been introduced.

15.4.3 Condensed and evaporated milk

Traditionally, condensed milk was bulk packed in barrels or tinplate containers. In India, sweetened condensed milk is the most popular out of all other concentrated milks and is packed in conventional food cans with double seam ends. Evaporated milk is recently packed in aseptic tetrapaks.

15.4.4 Butter

Because of high moisture content, butter, unlike solid fats is susceptible to mold growth. Flavor and odor are easily affected by absorption from other materials or through spoilage of butter due to oxidative rancidity. The package should, therefore, be opaque and a high barrier against oxygen and foreign odors. The most commonly used butter wrap is the vegetable parchment paper of 45

gsm.

Recently, embossed aluminium foil backed parchment paper has been introduced for UV light protection and sales appeal. A popular packaging style in some countries is to use plastic cups and plastic tubs with lids in different shapes and sizes. For such applications, PP (Polypropylene) and ABS (Acrylo-Butadiene-Styrene) are widely used.

15.4.5 Ghee

Ghee needs to be protected from chemical spoilage and rancidity caused by oxygen, light, heat, moisture and metal ions. A major portion of ghee was packed in lacquered or un-lacquered tinplate containers. Alternate packages, which are plastic based, are now gradually replacing tins. Ghee is also marketed in lined cartons with flexible laminated plastics as inner liner materials and in tetrapaks. In both these packs long shelf-life is achieved. Laminated pouches of metalized polyester based films are also used. For packaging of ghee, laminates of polyester, Nylon-6 and use of high barrier materials such as Ethylene Vinyl Alcohol (EVOH) polymer films with a trade name of EVAL can also be explored, as these materials could provide a fairly long shelf-life.

15.4.6 Milk powder

Milk powder is hygroscopic in nature and has a tendency to gain moisture from the atmosphere, which results in lumping or caking of the powder. Whole milk powder is highly sensitive to oxygen as well. Presence of oxygen causes spoilage of the product due to oxidation and rancidification, therefore the packages are required to be vacuum or nitrogen flushed. Milk powder is bulk packed in 25 kg capacity multiwall paper sacks with plastic liner made of polyethylene. Alternatively, the polyethylene liner can be laminated directly to the inner wall of the paper sack.

The flexible materials have evolved through polyethylene bags to sophisticated multi-ply laminates. Stand–up pouches of metalized polyester/LLDPE laminates and polyester/LLDPE laminates are used for skimmed milk powder. For whole milk powder, a typical structure for a plastic pouch is 12 µ polyester/9 µ Al foil/50 µ PE, and when gas flushed, these pouches are found to be as effective as canning to prolong shelf-life of milk powder. Latest development is the increasing use of pouches made from co-extruded film of LLDPE–Nylon–LLDPE with gas flushing and laminates of Polyester/Al foil/surlyn/Pd catalyst/surlyn, as oxygen scavenger.

15.4.7 Ice-cream

Conventional form of packages include paperboard cartons, paper cups and in some cases even metal containers. The various types of packages for ice-cream include:

- Paper board carton which is poly coated is poly-coated
- Thermoformed/injection moulded plastic containers made from HIPS (high impact polystyrene), PP (Polypropylene) or HDPE (high density polyethylene). The materials used for the lids are LDPE (low density polyethylene) or PS (polystyrene). The lids are of snap on type.
- Laminates of BOPP (biaxially oriented polypropylene) or PET (polyethylene terephthalate) are used for candies.

15.5 Malted Milk Food

Malted milk food is highly sensitive to moisture and is prone to oxidative changes in the presence of light, heat and oxygen. Aroma retention of the product and prevention of moisture and oxygen ingress, therefore, is very critical in protecting the product, and in selection of the right packaging material. The types of packages used conventionally are glass jars, tinplate containers, which are now slowly being replaced by plastic containers and flexible laminated pouches

15.5.1 Cheese

Cheese needs to be protected against moisture loss and ingress of oxygen in order to maintain the desired quality characteristics. In India, the traditional package of cheese is a hermetically sealed printed tin-plate container. Today, the flexible packaging films and laminates generally used for packaging of cheese to provide adequate moisture and oxygen barrier properties and to retain the vacuum are:

- Co-extruded LLDPE – TIE – Nylon – TIE – LLDPE
- Co-extruded LLDPE – TIE – EVOH – TIE – LLDPE
- Co-extruded film based on PVDC as the core material
- Laminates of metalized polyester/co-extruded nylon based film

15.5.2 Dahi/Yoghurt

Yoghurt has a very short shelf-life at room temperature. The traditional pack so far was the earthenware pot with a loose cover of glassine (a very thin and smooth paper that is air and water resistant) or greaseproof paper. The earthenware pots are very heavy, easily breakable and because of oozing of water from its body, the product inside develops shrinkage cracks. Recently, injection molded polystyrene and polypropylene cups have been introduced with aluminium foil based peelable lids.

15.5.3 Traditional dairy products

Traditionally, indigenous products have been packed in leaves, paper cartons or paper-board boxes. These materials do not provide sufficient protection to the product from atmospheric contamination and manual handling. Consequently, the sweets soon lose their typical body and texture, absorb foreign odor and lose their aroma characteristics and show mold growth, when products are stored in open metal trays.

Use of coated films, laminates of aluminium foil with various substrates, metalized films and combinations of various packaging materials need to be tested for suitability for these products. For instance, products like, gulabjamun and rasgolla need protection from light, oxygen, ingress or egress of moisture and micro-organisms; lacquered tinplate can is the most protective material, but this is very expensive.

15.6 Milk Capping

The well-proven method of bottle capping using aluminium foil introduced hygiene and high speed mechanization to milk packaging and contributed to one of the most efficient forms of re-used pack. The used glass bottles are collected and re-filled again and again – so saving valuable material resources. The compatibility of aluminium foil with heat-seal lacquers coupled with the

metal's excellent heat conductivity and stability makes it the ideal material for capping and heat-sealing of all types of plastic milk containers. Whether the milk is fresh, aseptically filled or sterilized in the container, the foil/coating combination can be designed to meet the demands of processing and distribution.

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Lesson 16
SOFT AND HARD WATER, TEMPORARY AND PERMANENT HARDNESS

16.1 Introduction

Water is a basic renewable natural resource upon which the survival and well being of living organisms depend. Quantity, quality and availability of water are critical factor in supporting our human civilization and standards of living. Man and animals enter hydrologic cycle as users of water and producers of waste in many forms. Water is also essential to the food supply and habitat of all other living organisms. The food industry uses large quantities of water. It is the most necessary item of the food industry. In addition to use in the growing of the raw products, water is used for generating steam, cleaning, peeling, grading, and conveying products, as a heat exchange medium in heating and cooling operations, for cleaning plant and equipment, for condensing vapors, for the fire protection, sanitizing, drinking, humidification, as an ingredient in the finished products and as a means of waste disposal. To successfully fulfill this versatile role in food industry, water must be in adequate supply, safer and of high quality. Moreover due to the globalization and implementation of WHO and ISO concepts the quality of raw as well as finished products has become very important. In the present scenario the management of water, both qualitatively and quantitatively is gaining importance. Therefore, the supply of adequate quantity and safe quality of water is of vital importance to the food industry particularly to dairy industry. Management of water in food industry consists of three components: i) quality at entry level, ii) water conservation and iii) waste management.

16.2 Types of Water

Generally water is classified into two categories i) Hard water and ii) Soft water. Water hardness is basically due to the presence of di- cations including Ca2+ and Mg2+. These ions enter a water supply by leaching from minerals. Water hardness is further of two types: i) Temporary hardness and ii) Permanent hardness. Temporary hardness is caused by the carbonates and bicarbonates of calcium and magnesium. This can easily be removed by boiling of water. On the other hand, presence of sulfates and chlorides of calcium and magnesium are responsible for permanent hardness of water. This kind of hardness is not removed by simple boiling but requires some complex operations. The combined effect of temporary and permanent hardness is called as total hardness of the water. Temporary hardness and permanent hardness are also known as ***carbonate hardness and non- carbonate hardness***, respectively. Conventionally hardness is expressed in terms of ppm of calcium carbonate. In industry, the major problem caused by hard water is the deposition of scales in and on the pipes which can clog plumbing and interfere with heat exchangers. These scale, are composed mainly of calcium carbonate (CaCO3), magnesium hydroxide [Mg(OH)2], and calcium sulfate (CaSO4). Calcium and magnesium carbonates tend to precipitate out as hard deposits to the surfaces of pipes and heat exchanger surfaces. This is principally caused by thermal decomposition of bi-carbonate ions but also happens to some extent even in the absence of such ions. In boilers, the deposits act as an insulation that impairs the flow of heat into water, reducing the heating efficiency and allowing the metal boiler components to overheat. In a pressurized system, this can lead to failure of the boiler.

The following equilibrium reaction describes the formation of calcium carbonate scales

$CaCO_3 + CO_2 + H_2O \leftrightharpoons Ca^{2+} + 2HCO_3^-$

Hard water, form white precipitate (scum) with soap solutions, instead of producing lather. This

effect arises because the di- cations destroy the surfactant properties of the soap by forming a solid precipitate. A major component of such scum is calcium stearate.

$2\ C_{17}H_{35}COO^- + Ca^{2+} \rightarrow (C_{17}H_{35}COO)_2\ Ca$

The Indian standards for water quality tolerances for processed food industry are as

Table 16.1 Bacteriological tolerances

Sl. No.	Characteristics	Tolerances
1	Coliformbacteria, MPN index per 100 ml	<1
2	Standard plate count, per ml, (max)	50*
3	Proteolytic and lipolytic organisms combined count per ml (max)	5

*Not applicable in the case of cooling water and of hot water supplied in dairy industry.

Table 16.2 Physical and Chemical tolerances (BIS, 1981)

Sl. No.	Characteristics	Tolerances
i)	Color (Hazen units), *Max*	20
ii)	Turbidity (units), *Max*	10
iii)	Odor	None
iv)	pH	6.5 to 9.2
v)	Total solids, mg/L, *Max*	1000
vi)	Total hardness (as $CaCO_3$), mg/L, *Max*	600
vii)	Sulphate (as So_4), mg/L, *Max*	200
viii)	Fluoride (as F), mg/L, *Max*	1.5
ix)	Chloride (as Cl), mg/L, *Max*	250
x)	Cyanide (as CN), mg/L, *Max*	0.01
xi)	Selenium (as Se), mg/L, *Max*	0.05
xii)	Iron (as Fe), mg/L, *Max*	0.3
xiii)	Magnesium (as Mg), mg/L, *Max*	75.0
xiv)	Manganese (as Mn), mg/L, *Max*	0.2
xv)	Copper (as Cu), mg/L, *Max*	1-0
xvi)	Lead (as Pb), mg/L, *Max*	0.1
xvii)	Chromium (as Cr^{+6}), mg/L, *Max*	0.05
xviii)	Zinc (as Zn), mg/L, *Max*	15.0
xix)	Arsenic (as As), mg/L, *Max*	0.2
xx)	Nitrate (as N), mg/L, *Max*	20
xxi)	Phenolic substances as $C_6H_5(OH)$, mg/L, *Max*	0.001
xxii)	Cadmium (as Cd), mg/L, *Max*	0.01
xxiii)	Mercury (as Hg), mg/L, *Max*	0.001

Table 16.3 Tolerances for radioactivity

Sl. No.	Characteristics	Tolerances
i)	Alpha emitters, *μc/ml, Max*	10^{-9}
ii)	Beta emitters, *μc/ml, Max*	10^{-8}

Table 16.4 Additional tolerances for specific operations

Sl. No.	Characteristics	Tolerance for		
	Total hardness (as $CaCO_3$), mg/L, Max	Cooling	Washing, flushing and general purpose	Processing
i)		30*	30**	-----
ii)	Iron (as Fe), mg/L, Max	--	0.1	0.1
iii)	Manganese (as Mn) , mg/L, Max	---	0.1	0.1
iv)	Slime forming organisms	Absent	---	---

*For waters which are re-circulated and used. In once through and run to waste systems, carbonate hardness should be absent.
**Especially if used for washing with soap or other alkaline detergents.

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Lesson 17
SOFTENING OF HARD WATER

17.1 Introduction

Food processors almost always treat at least some of the water used in the plants, even if they are supplied by a municipal system. This is due to the special requirements for use in boilers, cooling towers and similar equipment. Treatment may be done to control corrosion and formation of scale on equipment, to remove turbidity caused by solids, to eliminate staining, odor and flavor problems, and to assure safety for consumption-to name a few. Satisfactory procedure for one water supply may be inadequate for another. Designing a water treatment system for a food plant must be considered on an individual plant basis. The importance of adequately testing the water to be treated to determine the best methods for a given plant must be stressed.

17.2 Turbidity-Solids Removed

Turbidity results from suspended particles in water. The particles may range in size from 100,000 millimicrons in diameter for fine sand to colloidal suspensions with particle sizes from 1 to 200 millimicrons. Silt with a particle diameter of about 10,000 millimicrons tends to settle out as sediment in quiescent after. To produce clear water, removal of particles in colloidal suspension is usually necessary. Since colloidal suspensions are relatively stable, a coagulant is used to cause aggregation of particles of sufficiently high density to promote settling out for clarification. Inorganic chemicals commonly used as coagulants are Ferric sulfate, Ferrous sulfate, Filter alum, Sodium aluminate. Rapid settling increases the efficiency of clarification, which can often be improved with the addition of a filter aid. Filter aids are chemicals which speed floc formation and settling.

17.2.1 Softening

Softening of water is done to remove the hardness of water due to minerals. Different methods for water softening are as:

17.2.1.1 Cold lime method

Many municipal water treatment plants use the cold lime softening method. In this process, calcium oxide (CaO) is added to the hard water to form calcium hydroxide, which reacts with magnesium and calcium bicarbonates and free CO2 to form insoluble calcium carbonate and magnesium hydroxide. Magnesium hydroxide is a good flocculating agent which aids in precipitat¬ing the calcium carbonate particles. This treatment will usually result in water with about 70 to 85 ppm of calcium (4 to 5 grains per gallon) when discharged from the final filtration unit. Sand and gravel filters are commonly used for removing the precipitated salts by the cold lime softening method. Process is based on the following reaction:

$$CO_2 + Ca(OH)_2 = CaCO_3 + H_2O$$

carbon dioxide — calcium hydroxide — calcium carbonate — water

$$Ca(HCO_3)_2 + Ca(OH)_2 = 2CaCO_3 + 2H_2O$$

calcium bicarbonate — calcium hydroxide — calcium carbonate — water

$$Mg(HCO_3)_2 + 2Ca(OH)_2 = Mg(OH)_2 + 2CaCO_3 + 2H_2O$$

magnesium bicarbonate — calcium hydroxide — magnesium hydroxide — calcium carbonate — Water

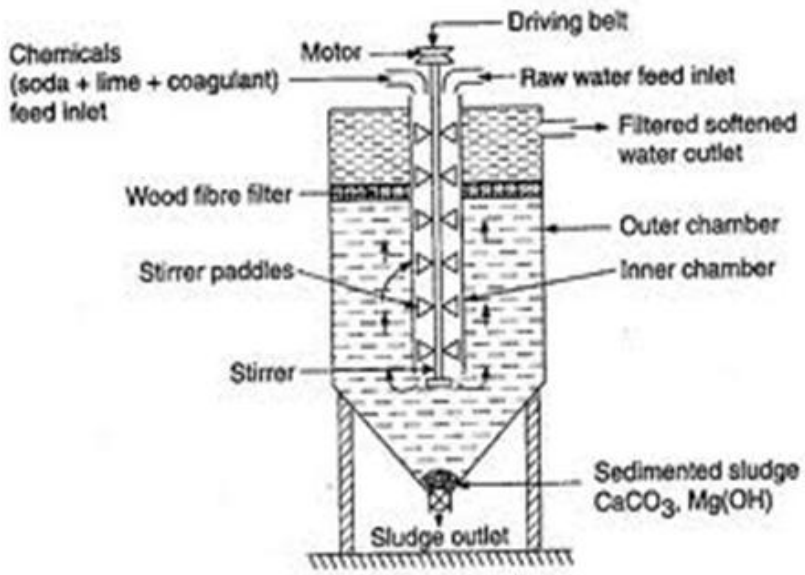

Fig. 17.1 Continuous Cold lime water softener

17.2.1.2 Base exchange softening method

Most food plants will find the base-exchange process to be a more practical and controllable method for softening the water for cleaning and other uses. The materials used in the ion-exchange are natural or synthetic zeolites which often are hydrous silicate or styrene based resins. In the sodium cation exchange, sodium from the zeolite or resin displaces an equivalent quantity of calcium and magnesium in the water as it passes through the bed. Sodium zeolite softening is the most widely applied use of ion exchange. In zeolite softening, water containing scale-forming ions, such as calcium and magnesium, passes through a resin bed containing SAC (Strong Acid Cation) resin in the sodium form. SAC resins derive their functionality from sulfonic acid groups (HSO_3^-).

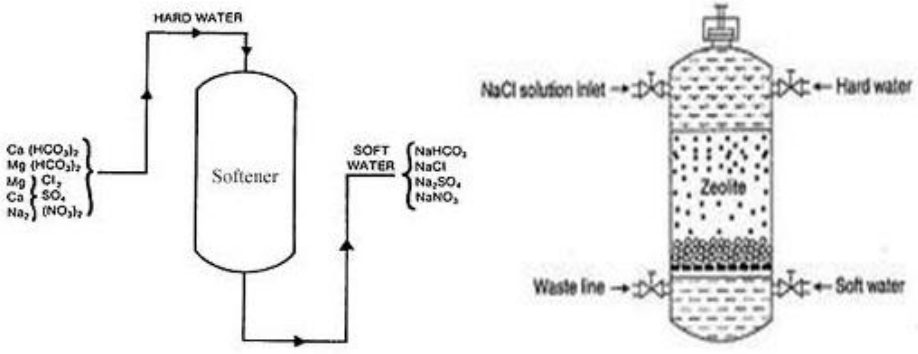

Fig. 17.2 Base-exchange softening method

In the resin, the hardness ions are exchanged with the sodium, and the sodium diffuses into the bulk water solution. The exchange reaction is reversible. When its capacity is exhausted, the resin can be regenerated with an excess of mineral acid. Strong acid cation exchangers function well at all pH ranges. The removal of hardness from water by a zeolite softening process is described by the following reaction:

$$CaSO_4 + Na_2Z \rightarrow Na_2SO_4 + CaZ$$

$$MgSO_4 + Na_2Z \rightarrow Na_2SO_4 + MgZ$$

$$CaCl_2 + Na_2Z \rightarrow 2NaCl + CaZ$$

$$Ca(HCO_3)_2 + Na_2Z \rightarrow 2NaHCO_3 + CaZ$$

In recent years the technology of ion exchange has advanced considerably and several excellent resins have been developed. For softening commonly used resins are of a sulfonated styrene divinylbenzene structure.

17.2.1.3 Demineralizing (Deionizing) water supplies

Although softening water with a sodium cycle ion-exchanger is most com¬monly found in processing plants, there is also need for demineralized (deionized) water for special purposes, such as use in the beverage industry. Several variations may be found in demineralization systems depending on the analysis of the untreated water and the desired purity of the treated water. Systems for demineralizing water are basically of two types, multi-bed and mixed-bed. Mixed-bed units offer the advantage of less space required, and they will also produce high quality water. Multi-bed and mixed-bed ion ex-changers are sometimes sequenced into a system to produce very high quality demineralized water.

17.2.1.4 Filtration

Filtering is almost invariably included in a water treatment system. In many cases, water is filtered before softening or demineralizing. Depending upon the system and quality of water desired, the final step may be filtration. Large water treatment plants for municipalities will often use gravity type filters. However, food processing plants will usually find the enclosed pressure type filters more satisfactory. Water may be passed through a series of filters each with a different filter media to achieve a special purpose. For removal of particulate matter sand and gravel filter is effective. Where low silica is desired, nonsiliceous anthracite is used instead of sand. Food plants will find activated carbon filters useful for improving the taste and odor of certain water supplies. These filters absorb phenols, chlorine and similar compounds. Filters with highly activated carbon require a special tank lining to protect the vessel from galvanic corrosion. Filter media are available for removing iron and manganese from water or to raise the pH of acidic water by removing carbon dioxide. The oxidizing filter medium which removes iron and manganese does so by forming an insoluble precipitate which collects on the bed. The precipitates are removed by periodic backwashing. Frequent regeneration of the bed with a solution of potassium permanganate restores the oxidizing capability for iron and manganese removal. A unit utilizing a rotary aerator and a bed of high luster anthracite coal as the filter media has the advantage of not requiring chemical treatment for regeneration. The unit appears useful for treating water with a high iron content and relatively low cost operation. Regular backwashing to expand the bed and remove the ferric precipitate is important as in any filter.

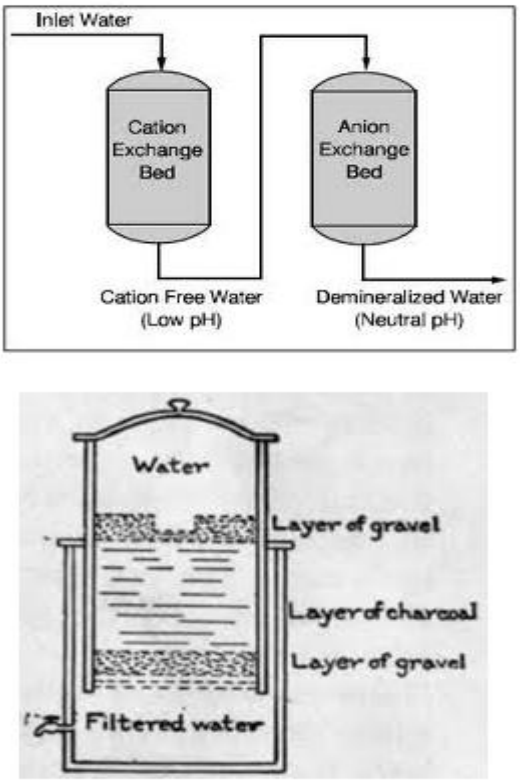

Fig. 17.3 water filter

17.2.1.5 Reverse osmosis systems

The technology of reverse osmosis (RO) is advancing rapidly. Reverse osmosis separates one component of a solution from another by placing the solution under pressure against a semi permeable membrane. Typically the pores of the semi permeable membranes used in reverse osmosis are 5 to 20 Angstrom units (5 to 20 x 10^{-8} cm) in diameter. A number of membranes have been developed, and cellulose acetate is on which is commonly used. Reverse osmosis is a method of purifying water to a high degree, especially when used in conjunction with a prefilter and an ion-exchanger. Some advantages cited for reverse osmosis water purification are: chemicals are unnecessary, membrane life is normally 1 to 3 years, low maintenance requirements, pressure is the only energy requirement, and membranes can be tailored for specific separations or where very high quality water is required.

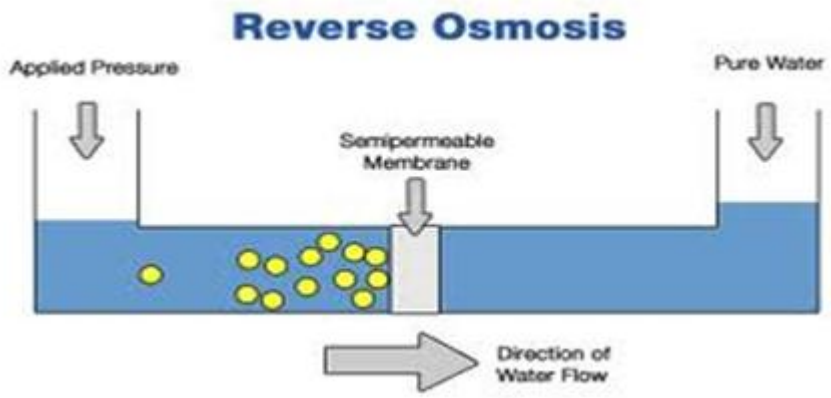

Fig. 17.4 Reverse osmosis

17.3 Chlorination of Water Supplies

Addition of small amounts of chlorine to water supplies acts as a safeguard against water-borne diseases. Food processing plants have increasingly been chlorinating water for plant use to improve sanitation. Chlorine may be added to water systems in food plants as a gas or as solution of chlorine compounds which are mainly hypo chlorites of sodium or calcium. Some plant operators have found chlorine dioxide to be very satisfactory where considerable organic matter is present, such as in recycled water systems.

Table 17.1 Chlorine Dose rates for specific purposes

Source/Puupose of Water	Dose rate (ppm)
Well	1-5
Surface	1-10
Cooling	3-5
Chilling	20
Washing	50

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Lesson 18
DETERGENTS AND SANITIZERS

18.1 Introduction

Milk is a good source of many nutrients like proteins, salts, lactose and vitamins, which are responsible for the fast growth of micro-organisms and scaling of heat exchangers etc. In dairy operations main processing treatment given to milk is heating which causes the scaling on the surfaces of heat exchangers and tubings. Milk traces, if left in the pipes and valves lead to the deterioration in the quality of milk. Therefore, the cleaning and sanitation of the dairy equipment is of utmost importance to meet the sanitary and phyto-sanitary requirements of the industry. The efficiency of cleaning and sanitization is directly related to the strength of the detergents and sanitizers used in the cleaning and sanitization purpose.

18.2 Detergents

A detergent is a substance which is (i) used to enhance the cleansing action of water

(ii) an emulsifier, which penetrates and breaks up the oil film that binds dirt particles,

(iii) capable of wetting surface(s) to allow it to penetrate the soil deposits and break

the soil into fine particles (deflocculation) and to hold them in suspension so that they do not redeposit on the cleaned surface(emulsification) (iv) must have good sequestering power to keep calcium and magnesium salts in solution.

There are two types of cleaning detergents: alkaline or acid that are often formulated with surfactants, chelating agents, and emulsifiers to enhance the effectiveness of the detergents. The most effective detergents in the dairy today are formulated with alkaline solutions that have chelators and surfactants. a wetting agent, which helps them to float off.

18.2.1 Alkaline detergents

The alkaline detergents are generally comprised of basic alkali, polyphosphates and wetting agents. None of the basic alkalis, higher phosphates or wetting agents can meet all the requirements of a good cleaner when used alone.

Basic Alkalis: The basic alkalis, such as soda ash, caustic soda, trisodium phosphate and sodium metasilicate form the bulk of most of the common dairy cleaners. Two or more of them are used in combination to overcome the weaknesses of a single compound and to give certain desirable properties to the blended product. For instance, Caustic soda is high in germicidal action and dissolving action on milk proteins, but it lacks deflocculating and emulsifying power as compared with other alkalis. In addition, caustic soda is objectionable in jobs requiring cleaning by hand because of hand burning as it is the most corrosive alkali.

Soda ash, is the most common constituent of dairy detergents today, and is the most inexpensive form of alkali. It is a poor water softener and has only fair deflocculating and emulsifying action. It has the advantage of being a good buffer. This makes it useful in solutions that are used over extended periods, as in hand bottle washing. When soda ash is used in hard water, calcium carbonate is precipitated and this precipitate causes hard water spotting and helps develop milk-

stone deposits on dairy equipment. This may be prevented in products containing soda ash by the addition of the higher phosphates in quantities large enough to sequester or tie up the water hardness. It is obvious that soda-ash cannot be used in large proportions in cleaners to be used in extremely hard water.

Trisodium phosphate, have become a very popular constituent in dairy cleaners because of its ready solubility and high deflocculating and emulsifying powers. It is a fair water softener because of the flocculent character and insolubility of the calcium and magnesium phosphates formed. When compared with metasilicate or soda ash, trisodium phosphate is also relatively corrosive on tin unless metasilicate is present as a protective agent in the mixture. Concentrations are sometimes limited to 0.5–1.5% to minimize phosphate levels in wastewater.

Sodium metasilicate, has high active alkalinity and excellent deflocculating and emulsifying properties. It, like trisodium phosphate, is only a fair water softener. The calcium and magnesium silicates formed in hard water are flocculent and insoluble in solutions. Although it is the strongest alkali next to caustic soda, it is relatively non-corrosive and has the property of protecting metals against corrosion by other alkalis. Metasilicate is very effective in holding the soil in suspension during the washing operation so that complete cleaning is possible.

18.2.2 Acid detergents

The use of acid detergents is commonly restricted to the removal of milkstone, water scale (calcium and magnesium carbonates). Acid detergents are more effective against bacteria than are alkaline detergents. The two most common types of acid detergents used are:-

18.2.2.1 Nitric acid

Nitric acid not only is used to remove milk-stone and other inorganic deposits, it also has biocidal properties when used either as a pure acid or in more stable, less hazardous mixtures with phosphoric acid. In addition, nitric acid attacks proteins. Nitric acid offers the added benefit of forming a protective layer of chromium oxide on the surface of food processing equipments made up of stainless steel which contains chromium, thus preventing the leaching of iron ions into the milk. Commercially available aqueous blends of 5-30% nitric acid and 15-40% phosphoric acid are commonly used for cleaning food and dairy equipment primarily to remove precipitated calcium and magnesium compounds (either deposited from the process stream or resulting from the use of hard water during production and cleaning). It should not be used in >1% concentration for stainless steel surfaces.

18.2.2.2 Phosphoric acid

Phosphoric acid is used widely as the basis of acid cleaning materials and finds greatest application in the removal of milk-stone and similar deposits on surfaces such as protein deposits. Its performance is greatly enhanced by adding an acid-stable surfactant, which promotes penetration of surface deposits and also assists in the process of rinsing at the end of the cleaning process. It often is used at a concentration between 2 and 3% w/v phosphoric acid for cleaning. Small quantities of complex organic acids are often added to enhance its effectiveness.

18.3 Sanitizers

According to United States Environmental Protection Agency (EPA) specifications, these are the compounds or type of antimicrobial that kills or irreversibly inactivates at least 99.9 percent of all

bacteria, fungi, and viruses (called microbials, microbiologicals, microorganisms) present on a surface. Most sanitizers are based on toxic chemicals such as chlorine, iodine, phenol, or quaternary ammonium compounds, and which (unlike some antiseptics) may never be taken internally. Sanitizers used in the dairies should have the following properties - i) non toxic ii) quick acting iii) non corrosive to hands and equipments iv) can be quickly applied and v) inexpensive.

The most commonly used sanitizers are the compounds of chlorine and iodine.

18.3.1 Chlorine compounds

These compounds include liquid chlorine, hypochlorites, inorganic chloramines, and organic chloramines. Chlorine-based sanitizers form hypochlorous acid (HOCl, the most active form) in solution. Chlorine compounds are broad spectrum germicides which act on microbial membranes, inhibit cellular enzymes involved in glucose metabolism, have a lethal effect on DNA, and oxidize cellular protein. Chlorine has activity at low temperature, is relatively cheap, and leaves minimal residue or film on surfaces.

18.3.1.1 Disadvantages

Chlorine compound is corrosive to many metal surfaces (especially at higher temperatures). Health and safety concerns can occur due to skin irritation and mucous membrane damage in confined areas. At low pH (below 4.0), chlorine (Cl_2) may interact with residual food components containing sulfur and form deadly mustard gas {bis(2-chloroethyl) sulfide}.

18.3.2 Iodine compounds

This sanitizer exists in many forms and usually exists with a surfactant as a carrier. These mixtures are termed as iodophors. The most active agent is the dissociated free iodine (also less stable). This form is most prevalent at low pH. Iodophors, like chlorine compounds, have a broad spectrum effect. These compounds are active against bacteria, viruses, yeasts, molds, fungi, and protozoans. These compounds are more specific against non- spore forming bacteria.

18.3.2.1 Disadvantage

Iodine is highly temperature-dependent and vaporizes at 120°F. Thus, it is limited to lower temperature applications.

18.3.3 Quaternary ammonium compounds (QACs)

Quaternary ammonium compounds (QACs) are a class of compounds which have the general structure as follows .

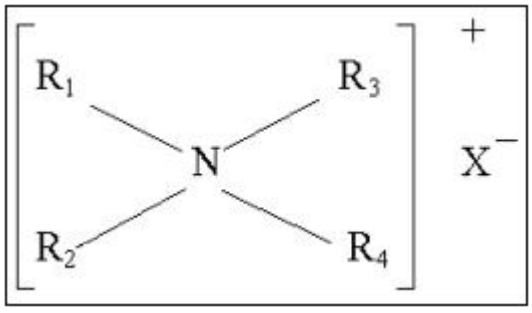

Fig. 18.1 General structure of Quaternary ammonium compounds

These compounds are generally positively charged cations, hence their mode of action is related to their attraction to negatively charged materials such as bacterial proteins. QACs are active and stable over a broad temperature range. Because they are surfactants, they possess some detergency. Thus, they are less affected by light soil than are other sanitizers.

18.3.3.1 Peroxyacetic acid (PAA)

This compound is known for its germicidal action, which remains for a long period. Peroxy acetic acid is relatively stable when used with a strength of 100 to 200ppm. This compound is free from phosphates and do not form foam when used. Another advantage of this compound is that it has low corrosiveness, tolerance to hard water, and favorable biodegradability. Peroxy acetic acid solutions are found to be useful in removing biofilms.

18.4 Determination of Sodium Hydroxide in Lye

Take 10 ml of concentrated lye solution in 100 ml volumetric flask. Dilute to 100 ml with distilled water. Take 10 ml dilute lye solution in conical flask. Add a few drops of phenolphthalein indicator. Titrate against 2.5 N hydrochloric acid till pink color disappears.
% NaOH in concentrated lye solution = Burette reading x 10

18.5 Analysis of Detergents and Sanitizers

18.5.1 Determination of nitric acid

Take 10 ml of concentrated sample of nitric acid in 100 ml volumetric flask. Dilute it to 100 ml with distilled water. Take 10 ml of the dilute sample in conical flask. Add a few drops of phenolphthalein indicator. Titrate against 0.1N sodium hydroxide solution till permanent light pink color appears.
% nitric acid in concentrated solution = Burette reading x 6.3

Derivation:

(Acid) = (Base)
$N_1V_1 = N_2V_2$
N_1 X 10 = 0.1 X V_2
N_1 (Dilute acid) = (0.1 x V_2)/10
N_1 (Undiluted) = (0.1* V_2 * dilution factor)/10
Where dilution factor = 10
Therefore,
N_1 = (0.1* V_2 * 10)/10
Strength of undiluted acid = (0.1* V_2 * 10 * Eq. wt. of acid)/10
Where eq. wt. of acid = 63
Therefore,
Strength of undiluted acid = (0.1* V_2* 10 * 63)/10
Strength of undiluted acid = V_2 x 6.3

18.5.2 Determination of available chlorine in sanitizer (Sodium Hypochlorite and Calcium Hypochlorite) solution

The method of determination of available chlorine is based upon the reaction between available

chlorine from hypochlorite solution and acidified potassium iodide solution in which iodine is liberated. The liberated iodine is then titrated against 0.1 N Sodium thiosulfate using starch as indicator. From the volume of sodium thiosulfate used, the quantity of available chlorine can be found out based on standard equation:
One ml of 0.1N Sodium thiosulfate = 0.003546 gm available chlorine.

Reaction:

I) If Sodium hypochlorite (NaOCl) is used as chlorine sanitizer

a) $[CH_3COOH + KI \longrightarrow CH_3COOK + HI] \times 2$

b) $NaOCl + 2HI \longrightarrow NaCl + H_2O + I_2$

By adding a and b

c) $NaOCl + 2KI + 2\ CH_3COOH \longrightarrow NaCl + 2CH_3COOK + H_2O + I_2$

d) $2Na_2\ S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$

II) If bleaching powder i.e. calcium hypochlorite ($CaOCl_2$) is used as chlorine sanitizer

e) $[CH_3\ COOH + KI \longrightarrow CH_3COOK + HI] \times 2$

f) $CaOCl_2 + 2HI \longrightarrow CaCl_2 + H_2O + I_2$

By adding e and f

g) $CaOCl_2 + 2KI + 2\ CH_3COOH \longrightarrow CaCl_2 + 2CH_3COOK + H_2O + I_2$

h) $2Na_2\ S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$

Take 5.0 ml of stock solution of sanitizer into a 250 ml volumetric flask. Make up the volume to the mark with distilled water and mix well. Pipette 50 ml of diluted solution in a 100 ml conical flask. Add 2 g of potassium iodide followed by 10 ml acetic acid. Titrate the solution against 0.1N sodium thiosulphate solution till the brown red color changes to straw yellow. At this stage add 1 ml of starch indicator and continue the titration till the blue color disappears. Note the titre value.

Available chlorine in sanitizer (ppm) = 3546 x burette reading.

Derivation:

1.0 ml of 0.1 N sodium thiosulfate = 0.003546 gm available chlorine. (Std. Eq.)
Suppose "V" ml of 0.1 N sodium thiosulfate is used for 50 ml of diluted sample
In other words:
50 ml of diluted sample requires = "V" ml of 0.1 N sodium thiosulfate solution.
250 ml of diluted sample requires = "V" x 250 ml of 0.1 N sodium thiosulfate solution.
50
Now, 250 ml of diluted sample is prepared from 5.0 ml of original stock solution.
Therefore, 5.0 ml of stock solution = "V" x 250 ml of 0.1 N sodium thiosulfate solution.
50
1.0 ml of stock solution = "V" x 250 ml of 0.1 N sodium thiosulfate solution.
50 5

= "V" X 250 X 0.003546 g of available chlorine
50 5
(Because, 1 ml of 0.1N Sodium thiosulfate = 0.003546 gm available chlorine)
Therefore, one million (106) ml of stock solution contains =
"V" x 250 x 0.003546 X 106 ppm of available chlorine

50 5
= 3546 x V ppm of available chlorine, where V is the burette reading

18.5.3 Determination of available iodine in sanitizer solution

The method of determination of available iodine in iodophore is based on the reaction of liberated iodine with sodium thiosulfate, forming sodium iodide and sodium tetrathionate. The end point of the reaction is indicated by the disappearance of the blue color produced by the solution with the starch indicator.

Take 5 ml of concentrated iodophor solution into 500 ml volumetric flask. Dilute it to 500 ml with distilled water. Take 10 ml of the dilute solution into conical flask. Add 30 ml chloroform and shake well, so that the pink color appears. Titrate against 0.01N sodium thiosulphate solution till pink color disappears.
% available iodine in iodophor = Burette reading x 1.27
ppm of available iodine in iodophor = % available iodine x 104

Derivation:

(Idophore) = (Sodium Thiosulfate)
$N_1V_1 = N_2V_2$
N_1 X10 = 0.01 X V_2
N_1 (Dilute iodophore solution) = (0.01 X V_2)/10
N_1 (Undiluted) = (0.01* V_2 * dilution factor)/10
Where dilution factor = 100
Therefore,
N1 = (0.1* V_2 * 100)/10

Strength of undiluted idophore = (0.01* V_2 * 100* eq. wt. of iodine)/10
Where eq. wt. of iodine = 127
Therefore,
Strength of undiluted idophore = (0.01 * V_2 * 100* 127)/10
Strength of undiluted idophore (g/litre) = V_2 x 12.7
% iodine in idophore = (V_2 x 12.7 x 100)/1000 = V_2 x 1.27

ppm of iodine in idophore solution = (V_2 * 1.27 * 10^6)/100

= V_2 x 1.27 x 10^4

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Lesson 19
SETTING UP QUALITY CONTROL LABORATORIES, TESTING FACILITIES AND MOBILE TESTING LABORATORIES

19.1 Introduction

In order to have an effective quality control programme, we need a well equipped quality control laboratory where we have to analyze the various materials, such as *raw materials*, *semi-finished*, *finished*, *stored products* etc. for various chemical and bacteriological parameters, so as to see that they meet the legal and quality standards.

Therefore, quality control laboratory should be regarded as one of the key features of any dairy plant. While designing a dairy plant, a due consideration should be given for the location and design of a quality control laboratory.

According to BIS, 5 categories (types) of laboratories have been suggested or recommended depending upon the quantity of milk handled by each dairy. However, these are recommendations only; one can make modifications in them to suit the requirements of a particular plant.

19.1.1 Types of dairy laboratories

1) Category A
For dairies which handle 1 lakh liters or more of milk per day.
2) Category B
For dairies which handle 25,000 litres or more of milk per day, but less than 1 lakh litres.
3) Category C
For dairies which handle 10,000 liters of milk or more per day, but less than 25,000 liters.
4) Category D
For dairies which handle 5,000 liters of milk or more per day, but less than 10,000 liters.
5) Category E
For dairies which handle less than 5,000 liters per day.

i) For the first three categories more or less similar facilities are required. The facilities are as follows:

Bacteriological room, sterilization and Media room, balance room, wash-up room, gas chamber, fume chamber (Fume hood or fume cupboard), Fine instrument room, Separate rooms for ***quality control officer and laboratory staff, working benches*** etc.

ii) For last two categories, fewer facilities are recommended. For example, Bacteriological room, Sterilization & Media room, fine instrument room are not generally required. These labs look like labs only for collection centers.

19.1.2 Location of QC lab in a dairy

Some important points for location are

1) It should not be at a remote (far-off) place from the dairy operations because procurement of samples or conveying of results should not take unnecessary amount of time.
2) At the same time, it should not be very close to the dairy operations where there can be lot of noise, vibrations, steam etc. In other words, it should be free from noise and vibration.
3) It should preferably be away from boiler houses.
4) It should be easily accessible for all the major activities of the dairy.

19.1.3 Layout/design of the laboratory

1) Laboratory should have adequate number of rooms/chambers and benches etc of suitable dimensions.
2) While setting up a laboratory, the possible expansion of the dairy should be kept in view.
3) Every laboratory should be provided with a minimum of 2 exits.
4) For each category (A, B, C, D, E) of lab, BIS has given two alternate layout plans.

19.1.4 General requirements for a QC lab

1) Walls

Walls of the lab should be smoothly finished. For example, they may be tiled with glazed tiles particularly in the wash up room and on the platform where the Gerber test is conducted.

2) Windows

There should be sufficient number of windows fitted with glass panes to receive adequate natural light.

3) Lighting

i) There should be adequate satisfactory natural day light.
ii) Northern or east - northern exposure is preferred for a satisfactory natural day light, particularly for color matching etc.
iii) Lights should be provided to give a minimum average intensity of 400 to 450 lumen/m^2 at working level.

Note

Instruments are available for measuring the intensity of light, like the one used in cricket grounds etc.

iv) Direct sun light should be avoided particularly for sensitive instruments (e.g. Chemical balance)
v) A roof light is helpful.

4) Flooring

(i) Flooring should be non- slippery.

(ii) Flooring should be capable of cleaning easily.

5) Store room

There should be two store rooms.
(a) One for acids, alkalies and ammonia
(b) Second for fine chemicals, glasswares, apparatus and equipments.

6) Benches

There are several designs/sizes of benches. Some of the examples are as:

- 150 x 75 x 90 cm
- 220 x 75 x 90 cm
- 360 x 67.5 x 90 cm
- 150 x 75 x 75 cm
- 210 x 75 x 75 cm

Any convenient size can be used.
Bench top should be made of material resistant to acid and alkali (For example, acid and alkali proof tiles or sunmica may be used).

A. Above bench fittings

Shelves should be provided above the bench to keep various solutions, chemicals, reagent bottles, glassware etc.

B. Under Bench fittings

The drawers and cupboard with shelf accommodation should be provided. The under bench unit could be of fixed or removable type and inter changeable.

7) Cupboards

Lab should have adequate number of cupboards - not only to protect the apparatus but also to give neat and clean appearance. For this, wall cupboards are preferred which provide space for storing some of the material without occupying the floor space.

8) Sinks

A. Sinks of suitable dimensions should be provided in wash-up rooms. They may be made of glazed earthenware or vitreous ware or stainless steel. Preferable dimensions of sinks for wash-up room are 75 x 45 x 25 cm.

B. Sinks for Service bench or laboratory bench or working bench should be fitted beneath (i.e. by the side of) the bench top. They may be made of porcelain or other suitable material. Dimensions for such sinks are 45 x 30 x 20 cm.

9) Fume cupboard/ fume hood/fume chamber

There should be a fume cupboard provided with an efficient means of removing objectionable

fumes, gases, vapors etc for carrying out operations which cause fumes etc. Fume chambers should have the provision for gas, water, waste outlet and electricity.

Fig. 19.1 Fume hood

10) Water supply

There should be adequate water supply.

11) Distilled water plant

Distilled water plant of adequate capacity should be available in each lab (Fig 19.2).

Fig. 19.2 (a) Metal Distillation Assembly

Fig. 19.2 (b) Glass Distillation Assembly

Fig. 19.2 (c) Quartz Distillation Assembly

12) Gas

Each lab should have provision of gas supply, in the form of own gas supply, in the form of own gas plant or portable gas supply.

13) Electric supply

Each lab should have electric supply. Electric points for water bath, heaters oven etc. should be provided.

14) Service lines (of water, gas & electric power)

The service lines, namely, those of tap water, electric power and gas, should run along the walls, concealed 80 cm above floor level and connection taken to the laboratory benches.
While installing service lines, two main points should be kept in mind:

a. Benches should be easily removable.
b. Service lines should be easily accessible.

15) Drainage Underground drainage system should be there.

16) Fire-extinguishers

Laboratory shall be equipped with fire extinguishers of suitable sizes and first aid box.

17) Ventilation

To ensure sufficient or proper ventilation, an exhaust fan of suitable size should be provided.

18) Air-Conditioners

Each laboratory, as far as possible, shall be air – conditioned.

19) Chemicals, glassware & Consumable Stores

Laboratory should be equipped with necessary chemicals, glassware and consumable Stores.

20) Equipments

Laboratory should be equipped with necessary equipments.

19.1.5 List of equipments ordinarily required in QC lab

- Mojonnier apparatus, water baths

Fig. 19.3 Mojonnier flask and flask stand

- Hot air oven, Hot plates
- Analytical balances, Desiccators
- Colorimeter/Spectrophotometer
- Nitrogen digestion/distillation apparatus

- Glass distillation apparatus
- Refrigerator, BOD incubator
- Microscope, Autoclaves
- Milkotester/Milko scan
- Gerber fat testing centrifuge
- Automatic measure for Gerber acid and iso- amyl alcohol
- pH meter, Viscometer
- Pro-milk, Infra-red Moisture tester
- General purpose centrifuge
- Solubility index apparatus
- Butyro-refractometer with circulating water bath
- Polarimeter

19.1.6 Special Requirements for specific purposes

1. Fat Test

A white tiled platform of suitable dimension would be found to be useful for conducting fat test.

2. Wash-up Room

In wash up rooms, it is desirable to have 3 sink units with draining board and draining rack.

3. Balance Room

The balance room should be so located in laboratories that it is least affected by vibrations.

4. Doors

The doors of the laboratory should be self closing.

5. Bacteriological Room

The bacteriological room should be dust-free and air- conditioned.

6. Drains

The drains should be made of an acid-proof material. While constructing the drains, the volume of discharge should be taken into account. The drains should be provided with suitable traps at suitable places to prevent blockage of drains.

7. All the rooms mean for analytical work in the laboratory should be fly-proof and rodent proof.

19.1.7 Various chemical tests ordinarily conducted in a dairy laboratory

1. Organoleptic test
2. Sediment test
3. Alcohol test
4. COB test
5. Acidity

6. Fat
7. SNF
8. Freezing point determination
9. Adulterants
10. Preservatives
11. Neutralizers
12. Phosphatase test
13. Hardness of water
14. Available chlorine (Cl2)
15. pH
16. Turbidity test
17. Salt (NaCl) purity
18. BR at 40°C
19. Reichert–Meissl value (RM) and Polenske value (PV)
20. Acid value
21. Sugar/lactose
22. Over-run (weight per liter)
23. Strength of alkali/acid etc.

19.2 Mobile Testing Laboratories

The mobile laboratory is the integrated analytical platform equipped with state-of–the–art measurement systems. It is to detect, analyze and confirm the chemical and biological adulterants/contaminants in food, water, milk etc. This lab in real sense is full-fledged traditional laboratory in the field as a mobile unit. However, in some cases it can be a mobile unit with limited tests in the field of food/ dairy analysis and depending upon the needs the laboratory can be designed. The mobile testing laboratory for the detection of food adulteration includes some rapid chemical and microbiological tests in particular. This lab should have proper safety features like pressurized air system, biological safety cabinet etc. These labs are fabricated on a specially modified mobile platform like heavy duty trucks. The concept of mobile testing laboratories is already in place in many developed countries as well as in some developing countries. In India, the newly formed Food Safety and Standards Authority (FSSAI) has taken an initiative to setup the mobile testing laboratories. These Mobile Labs are expected to be positioned at various locations in the country, including Delhi on the basis of risk assessment.

Fig. 19.4 Mobile testing

19.3 Objectives of Mobile Testing Laboratory

To take process of food testing to the door steps of the consumers and others.

i) The labs will visit the local markets, households, restaurants, godowns, schools and other public places, conduct tests on the spot and declare the result, thereby creating awareness among the people and also furnish feedback to the food safety authorities.

ii) Collection of information by means of testing and preparation of a data base, which can be useful in maintaining surveillance and could also be utilized by the Food Safety Officers, Laboratories and others involved in Food Safety regulation.

19.4 Design of the Laboratory

The mobile testing laboratory is generally constructed by laminated panels with excellent insulation properties. These panels are mounted in a galvanized steel frame. External dimensions generally depend upon the type and kind of testing to be carried out in a particular laboratory. The container is designed in such a manner that it can be lifted by both crane, truck with container hoist and can be transported by truck, ship, train and aeroplane. These laboratories can also be fitted in the heavy duty trucks. The laboratory can be divided into two portions internally by a sliding door. One half of the laboratory can be used for the preparation of samples and the other functions as a measurement laboratory where instrumentation can be located. The platform includes both rear and side entry doors with dual pan windows. The units should be equipped with adequate number of generators most likely two numbers one on each side of the platform. These are directly controlled from the outside or from a control panel located inside the laboratory compartment. Additionally there should be filtered fresh air intakes and exhaust vents with HEPA (High Efficiency Particulate Air) filters to avoid contamination of environment. A basic mobile laboratory should have the following features:

Fig. 19.5 Laboratory

19.4.1 Mobile laboratory

At least 20 ft. container made up of laminated aluminium panels with excellent insulation mounted in a galvanised steel frame. Ext. dimensions: 606 x 244 x 244 cm.

i) Provision for a connection to external power supply (230 - 400 V).
ii) Facilities for connection to external water supplies, air conditioning and internal climate control for operation in adverse weather conditions.
iii) Separate air conditioning and ventilation systems.
iv) Built in diesel generator for power supply in the event of power cuts.

v) Fume hood, refrigerator/freezer/microwave.
vi) Lines for telephony, data, internet, e-mail.

19.4.2 Equipment

Type of equipment depends upon the kind of testing to be performed. The possible equipments are as:
i) Sampling equipment and containers.
ii) Milko tester/Milk analyzer
iii) Ovens, Incubators.
iv) Gerber Centrifuge and clinical centrifuge.
v) Rapid chemical testing kit.
vi) Rapid microbiological testing kits
vii) Biosensor based tests for antibiotics etc.
viii) Lactometers,
ix) Computers (PC's), Printer
x) Protective equipment, electronic personnel.

Fig. 19.6 Equipments

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Lesson 20
CALIBRATION OF LABORATORY GLASSWARE: BUTYROMETER, PIPETTES, LACTOMETERS, THERMOMETER AND BURETTES

20.1 Introduction

In dairy plant, milk is critically analyzed for composition and quality during reception, processing and subsequent dispatch for sale. This is required for pricing, checking its suitability for processing and to know its compliance with the legal standards. In spite of the utmost care taken during analysis of milk and milk products, sometimes the results of the analysis can be erroneous due to use of inaccurately calibrated glassware. Therefore, it is very essential to check the calibration of glassware before being used for analysis.

20.2 Calibration of Milk Butyrometer

Calibration means establishing and recording the measurement uncertainty of measuring equipment. A true calibration does not involve any adjustment of an instrument but may demonstrate the need for adjustment. At the simplest level, calibration is a comparison between measurements-one of known magnitude or correctness made or set with one device and another measurement made in as similar a way as possible with a second device. The device with the known or assigned correctness is called the standard. The second device is the unit under test (UUT), test instrument (TI), or any of several other names for the device being calibrated.

20.2.1 Calibration of milk butyrometers

Following are some of the methods:

(i) Comparison method

This is not accurate method, however, sometimes and usually this method is mostly used. In this the accuracy of newly purchased butyrometers is compared by estimating fat by Gerber method in a one milk sample, along with the butyrometers of the previous batch, well calibrated and known to be accurate. If the readings of the fat values of new butyrometers are the same as of old ones, then the new butyrometers are accepted otherwise rejected. Limitation of this method is that the previous butyrometers may not be accurate or their internal volume may have been changed due to acid corrosion.

(ii) BIS Method

In this method a specially designed mercury pipette is used to calibrate the butyrometers. The method is based on the principle that the internal volume of the graduated tube of the milk butyrometer is 0.125 ml corresponding to each 1% fat range. In other words, the full scale of graduated tube from 0 to 10% fat marks, has the internal volume of 1.25 ml. Accordingly an automatic mercury pipette has been designed to dispense exactly 0.3125 ml mercury which fills the tube corresponding to 2.5% fat graduation limits. To calibrate the full scale from 0 to 10% fat marks, the bulb of the butyrometer is first filled upto 10% graduation mark as the base point. Then the mercury is added in the butyrometer from the mercury pipette four times, each time dispensing exactly 0.3125 ml of mercury corresponding to 2.5% fat graduation limit on the

butyrometer column. If the graduated column of the butyrometers are exactly filled from 0 to 10% fat marks in four deliveries of the mercury pipette, then the butyrometers are accepted, otherwise rejected.

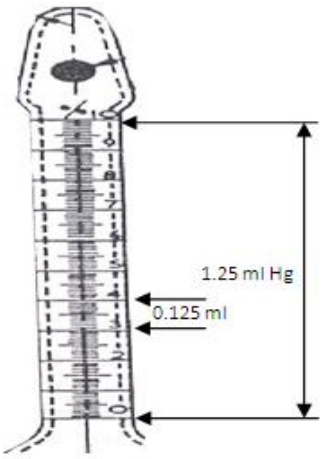

Fig 20.1 Butyrometer stem

The mercury is chosen as the filling liquid due to its following properties:

(a) It does not stick to the sides of the container, hence any error due to sticking of liquid at the unwanted area or sides of the butyrometers will not be there.

(b) It has very high density, therefore, a small change in volume, will be evidenced clearly by a great change in weight.

For cream butyrometer, calibration may be checked at each 10% graduation (at any three points). Here the internal volume of each 10% graduation is checked which should be 0.568 ± 0.004 ml.

For cheese butyrometer, calibration may be checked at each 5% graduation (at any three points). Here the internal volume of each 5% graduation is checked which should be 0.169 ± 0.002 ml.

20.2.2 Calibration of milk pipettes

Following are some of the methods:

(i) Comparison method

This is not accurate method, however, sometimes and usually this method is mostly used. In this the accuracy of newly purchased pipettes is compared by estimating fat by Gerber method in a one milk sample, along with the pipettes of the previous batch, well calibrated and known to be accurate. If the readings of the fat values of new pipettes are the same as of old ones, then the new pipettes are accepted otherwise rejected. Limitation of this method is that the previous pipettes may not be accurate or their internal volume may have been changed due to breakage of their tips.

(ii) BIS method

This method is based upon the definition of milk pipette as given by BIS. According to BIS the milk pipette is defined as to dispense 10.75 ± 0.03 ml of distilled water at 27°C when held for 15 s. For calibrating the milk pipettes, the water dispensed by the pipette is taken in a previously weighed beaker and its mass is recorded. Knowing the density of the water at 27°C i.e. 0.99654, the volume of the water dispensed is calculated as

Volume of water = (Mass of water dispensed)/(0.99654)
If the calculated volume of water dispensed by the pipette is equal to 10.75 ± 0.03 ml, then the pipette is accepted, otherwise rejected.

(iii) Mathematical method of calibration and graduation

For checking the marks/points corresponding to 10.75 ml capacity of milk pipettes, the following procedure can be applied:

As the stem of the pipette is of uniform cross-section, therefore, internal volume per unit length of stem at any point is constant. Fill the pipette with distilled water to a temporarily marked point A on the upper stem of the pipette and dispense in a tared beaker and weigh. Knowing density of water, calculate the volume of pipette up to the mark A. Similarly again fill up the pipette with the water up to another point B (above A) and find the volume of water dispensed.

Let volume of water up to point A = Va ml
And volume of water up to point B = Vb ml
Therefore, volume between A and B = (Vb – Va) ml
Now note the distance between A and B points, let it is = d cm
Therefore, stem of the pipette has volume = (Vb – Va)/d ml per cm length.
Now, let Va is less than 10.75 ml
Therefore, difference of volume = (10.75 – Va) ml
Now as we know that volume (Vb – Va)/d ml occupies length = 1 cm
And (10.75 – Va) ml occupies length = d x (10.75 – Va)/(Vb – Va) cm
Therefore, mark a point above A at a distance = d x (10.75 – Va)/(Vb – Va) cm, which will correspond to 10.75 ml mark.

20.2.3 Calibration of lactometers

Lactometer, which works on the Archimedes principle, is basically a specific gravity (sp. gr.) hydrometer specifically designed for milk. Stem of the BIS lactometer has graduation range of 20-35.

Fig. 20.2 BIS lactometer

Its calibration can be checked by the following methods:

(i) Comparison method

In this method each lactometer is calibrated before use by floating side by side in a liquid, against a standard lactometer. If the lactometer readings of newly purchased lactometers resemble with that of standard lactometer, then the new lactometers should be accepted otherwise rejected.

(ii) BIS method of testing accuracy of BIS lactometer

Dissolve appropriate mass of anhydrous sodium carbonate given below in 300 ml of distilled

water. Add 50 ml 92% ethanol to the solution so obtained and make up the total volume of 500 ml with distilled water. Compare the accuracy of each and every lactometer in all the solutions.

Table 20.2 Specific gravity and lactometer reading of pure Sodium carbonate Solution

Sl. No.	Wt. of anhydrous Na_2CO_3 (gm/500ml)	Specific gravity at 27°C (gm/ml)	Lactometer Reading (LR)
1	19.2	1.025	25
2	21.6	1.030	30
3	24.0	1.034	34

(iii) BIS method of testing accuracy of Quevenne lactometer

This can be conveniently done by taking the specific gravity of suitable salt solutions at 15.5°C. The solutions used are given in Table 20.3.

Table 20.3 Specific gravity of pure Sodium Chloride Solution

Sl. No.	Pure sodium chloride solution	Specific gravity at 15.5°C (gm/ml)	Lactometer Reading (LR)
1	3.863 %	1.026	26
2	4.415 %	1.032	32

The sp. gr. of these salt solutions must be checked by a sp. gr. bottle, and then the lactometer readings are taken in exactly the same way as with milk.

Fig.20.3 quevenne lectometer

20.2.4 Calibration of thermometers

There are several types of thermometers depending upon the types of temperatures and their measuring ranges. In milk testing glassware, mainly two types of thermometers are used- (a) thermometer (range 0 to 100°C) and (b) freezing point depression thermometer (range –0.5 to 0°C). Following are the methods to calibrate these thermometers:

20.2.4.1 Calibration of 0-100°C thermometer

(i) Comparison method

Though it is not very accurate method, however, usually it is mostly used. In this thermometers are compared with one or more thermometers which are known to be accurate. At least two different temperature marks (preferably nearer to lower and upper limits) are compared.

(ii) Physical method

In this method the zero point is located by dipping the mercury bulb of the thermometer in the melting ice kept in a wide funnel. Where the temperature becomes stationary, mark that 0°C point. The 100°C point is located by keeping the mercury bulb of the thermometer in steam at normal pressure. If the pressure is not 760 mm then appropriate correction is applied. In general at the natural atmospheric pressure 0.038°C is added or deducted from the observed boiling point per mm pressure of mercury lower or higher than 760 mm, respectively.

20.2.4.2 Calibration of freezing point depression thermometer

Before calibration of these thermometers, the definitions of two types of solutions are to be understood

1 Molar solution

It is defined as a solution prepared by dissolving one gm molecule of any substance in solvent so as to make 1000 ml solution.

1 Molal solution

It is defined as a solution prepared by dissolving one gm ion (in case of ionized molecules) or molecule (in case of non-ionized molecules) in 1000 gm of solvent.

The soluble ions or molecules play very important role in depressing the freezing point of solvent such as water. Milk is a solution in which water is the solvent and mainly lactose and minerals are solutes, which depress its freezing point. The one molal solution of any ion or unionized molecule depresses the freezing point of water by 1.86°C. It means one molal solutions of non-ionized substances like sugar (360g in 1000g of water), glucose (180 g in 1000 g of water) etc. depress the freezing point by 1.86°C. However, one molal solution of ionized molecules depresses the freezing point by (1.86 x numbers of ions per molecules)°C. For example, one molal solution of sodium chloride (Na+Cl-) will depress the freezing point by 1.86 x 2 = 3.72°C.
Now, 1.86°C is depressed by a solution = 1 molal

1°C is depressed by a solution = 1/1.86 = 0.538 molal
0.5°C is depressed by a solution = 0.269 molal

Therefore, -0.5°C and -1°C points on the freezing point depression thermometer are checked by using the 0.269 and 0.538 molal solutions, respectively.
For pure milk –0.54°C = 0.29 molal or –0.55°C = 0.3 molal.

20.2.5 Calibration of burette

Burette is checked at various intervals say 5, 10, 25 or 50 ml for determining the accuracy of its scale and the capacity of the burette. The volume actually delivered for each interval is obtained as per the procedure outlined for milk pipette.

Table 20.4 Tolerance for burette

Capacity (ml)	Burettes	
	Class A	Class B
1	0.006	0.01
2	0.010	0.02
5	0.010	0.02
10	0.020	0.05
25	0.050	0.10
50	0.050	0.10
100	0.10	0.20

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Lesson 21
ACCREDITATION OF ANALYTICAL LABORATORIES

21.1 Introduction

Accreditation is a procedure by which the accrediting body gives formal recognition that a body or organization is competent to carry out specific tasks. The concept of laboratory accreditation was developed to provide a means for third-party certification of the competence of laboratories to perform specific type(s) of testing and calibration. Laboratory accreditation provides formal recognition to competent laboratories, thus providing a ready means for customers to identify and select reliable testing, measurement and calibration services. To maintain this recognition, laboratories are re-evaluated periodically by the accreditation body to ensure their continued compliance with requirements, and to check that their standard of operation is being maintained. Laboratory accreditation uses criteria and procedures specifically developed to determine technical competence. Specialist technical assessors conduct a thorough evaluation of all factors in a laboratory that affect the production of test or calibration data. The criteria are based on an international standard called International Organization for standardization/ International Electrochemical Commission (ISO/IEC) 17025, which is used for evaluating laboratories throughout the world. Government of India has authorized National Accreditation Board for Testing and Calibration Laboratories (NABL) as the sole accreditation body for the purpose of accreditation of Testing and Calibration laboratories. NABL is an autonomous body under the aegis of Department of Science and Technology, Government of India, and is registered under the Societies Act. NABL has been established with the objective to provide Government, Industry Associations and Industry in general with a scheme for third-party assessment of the quality and technical competence of testing and calibration laboratories. NABL is a full member of both ILAC (International Laboratory Accreditation Cooperation) and APLAC (Asia Pacific Laboratory Accreditation Cooperation).

21.2 Process of Accreditation

21.2.1 Stage I

- Prepare your laboratory's application for NABL accreditation, giving all desired information and enlisting the test(s) / calibration(s) along with range and measurement uncertainty for which the laboratory has the competence to perform. Laboratory can apply either for all or part of their testing/calibration facilities. Formats NABL 151, NABL 152 & NABL 153 are to be used by Testing, Calibration & Medical Laboratories respectively for applying to NABL for accreditation.
- Laboratory has to take special care in filling the scope of accreditation for which the laboratory wishes to apply. In case, the laboratory finds any clause (in part or full) not applicable to the laboratory, it shall furnish the reasons.
- Laboratories are required to submit three sets of duly filled in application forms for each field of testing/calibration along with two sets of Quality Manual and Application Fees.
- NABL Secretariat on receipt of application will issue acknowledgement to the laboratory. After scrutiny of application for it being complete in all respects, a unique Customer Registration Number will be allocated to laboratory for further processing of application.
- NABL Secretariat shall then nominate a Lead Assessor for giving Adequacy Report on the Quality Manual/Application submitted by the laboratory. A copy of Adequacy Report by

Lead Assessor will be provided to Laboratory for taking necessary corrective action, if any. The laboratory shall submit Corrective Action Report.

- After satisfactory corrective action by the laboratory, a Pre-Assessment audit of the laboratory will be organized by NABL. Laboratories must ensure their preparedness by carrying out its internal audit before Pre-Assessment.

21.2.2 Stage II

- NABL Secretariat shall organize the Pre-Assessment audit, which shall normally be carried by Lead Assessor at the laboratory sites.
- The pre-assessment helps the laboratory to be better prepared for the Final Assessment. It also helps the Lead Assessor to assess the preparedness of the laboratory to undergo Final Assessment apart from Technical Assessor(s) and Total Assessment Man-days required vis-à-vis the scope of accreditation as per application submitted by the laboratory.
- A copy of Pre-Assessment Report will be provided to laboratory for taking necessary corrective action on the concerns raised during audit, if any.
- The laboratory shall submit Corrective Action Report to NABL Secretariat.
- After laboratory confirms the completion of corrective actions, Final Assessment of the laboratory shall be organized by NABL.

21.2.3 Stage III

- NABL Secretariat shall organize the Final Assessment at the laboratory site(s) for its compliance to NABL Criteria and for that purpose appoint an assessment team.
- The Assessment Team shall comprise of a Lead Assessor and other Technical Assessor(s) in the relevant fields depending upon the scope to be assessed.
- Assessors shall raise the Non-Conformance(s), if any, and provide it to the laboratory in prescribed format so that it gets the opportunity to close as many Non-Conformance(s) as they can before closing meeting of the assessment.
- The Lead Assessor will provide a copy of consolidated report of the assessment to the laboratory and send the original copy to NABL Secretariat.
- Laboratory shall take necessary corrective action on the remaining Non-Conformance(s)/other concerns and shall submit a report to NABL within a maximum period of 2 months.

21.2.4 Stage IV

- After satisfactory corrective action by the laboratory, the Accreditation Committee examines the findings of the Assessment Team and recommends additional corrective action, if any, by the laboratory.
- Accreditation committee determines whether the recommendations in the assessment report are consistent with NABL requirements as well as commensurate with the claims made by the laboratory in its application.
- Laboratory shall have to take corrective action on any concerns raised by the Accreditation committee.
- Accreditation committee shall make the appropriate recommendations regarding accreditation of a laboratory to NABL Secretariat.
- Laboratories are free to appeal against the findings of assessment or decision on accreditation by writing to the Director, NABL.
- Whenever possible NABL will depute its own technical personnel to be present at the time of assessment as Coordinator and NABL observer. Sometimes, NABL may at its own cost

depute a newly trained Technical Assessor as "Observer" subject to convenience of the laboratory to be assessed.

21.2.5 Stage V

- Accreditation to a laboratory shall be valid for a period of 2 years and NABL shall conduct periodical Surveillance of the laboratory at intervals of one year.
- Laboratory shall apply for Renewal of accreditation to it at least 6 months before the expiry of the validity of accreditation.

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