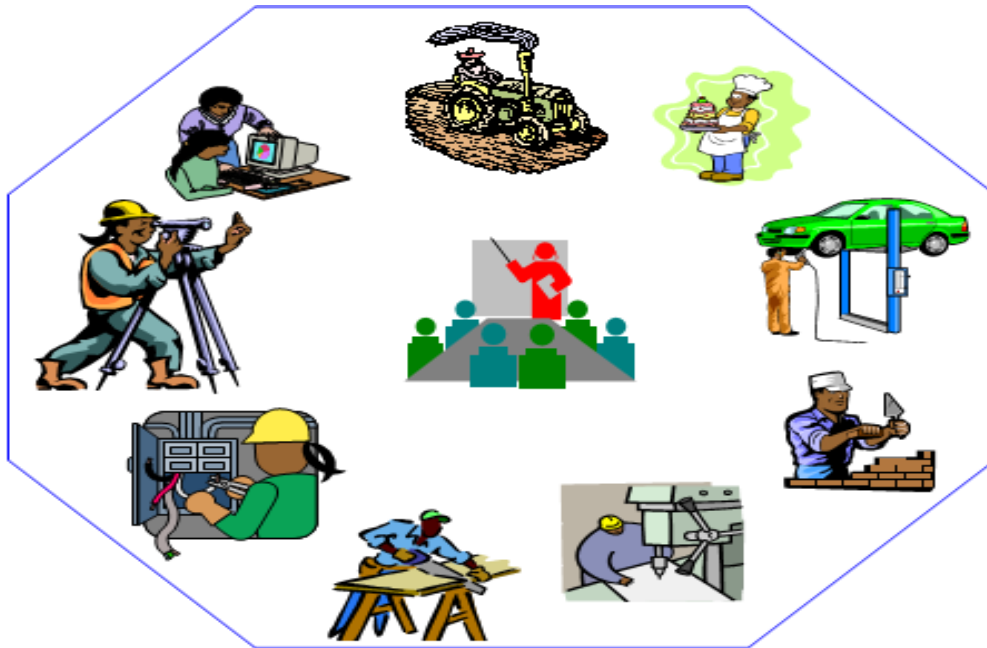




Dairy Products Processing

Level-III

Based on October 2019, Version 2 OS and
March 2021, V1 Curriculum



**Module Title: - Carrying out Sampling and Interpret
Tests Results for dairy products**

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LG #12

LO #1 Implement sampling procedures in dairy products processing

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Identifying and documenting sampling points for physical, chemical and microbial properties
- Determining an appropriate sampling size
- selecting and sterilizing sampling tools and equipment
- documenting and implementing the sampling plan or sampling requirement
- Implementing safety hazards and control methods, legislations and policies and procedures

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Identify and document sampling points for physical, chemical and microbial properties
- Determine an appropriate sampling size
- select and sterilize sampling tools and equipment
- document and implement the sampling plan or sampling requirement
- Implement safety hazards and control methods, legislations and policies and procedures

Learning Instructions:

- 1 Read the specific objectives of this Learning Guide.
 - 2 Follow the instructions described below.
 - 3 Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
 - 4 Accomplish the “Self-checks” which are placed following all information sheets.
 - 5 Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
- If you earned a satisfactory evaluation proceed to “Operation sheets
 - Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
 - If your performance is satisfactory proceed to the next learning guide,
 - If your performance is unsatisfactory, ask your trainer for further instructions or go back to “Operation sheets”.



Information Sheet 1- Identifying and documenting sampling points for physical, chemical and microbial properties

1.1. Introduction

The Dairy Food Standards are aimed at protecting consumers' health and ensuring fair practices in the food trade. The prescribed method of sampling are designed to ensure that fair and valid sampling procedures are used when dairy food is being tested for compliance with a particular commodity standard.

The sampling methods are intended for use as methods designed to avoid or remove difficulties which may be created by diverging legal, administrative and technical approaches to sampling and by diverging interpretation of results of analysis in relation to lots or consignments of foods, in the light of the relevant provision(s) of the applicable standard. A lot is a definite quantity of some commodity manufactured or produced under conditions, which are presumed uniform for the purpose of these Guidelines.

1.2. Purpose Of Sampling

Label should mention purpose of drawing / collecting the sample i.e. whether it's for regulatory purpose or for monitoring only.

1.3. Regulatory

Regulatory samples are picked for evaluation in case of certain concerns, issues. The reports of these analyses are filed for legal actions. Keeping this in mind, the sample integrity, homogeneity, and representativeness is vital for a fair and meaningful inference and subsequent actions.

Formal samples will be taken where formal enforcement action may result if an adverse report is received following examination or analysis. Hence formal samples have to be purchased or procured by suitably trained, qualified and experienced authorized officers. The officer should strictly use the procedure for statistical sampling.

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The objective of the sampling procedures is to ensure that any sample procured is a 'fair sample' that accurately reflects the constituents of the bulk material being sampled.

1.4. Monitoring

For samples to be drawn for the monitoring purpose, the notified institutions / labs should be involved as per approved protocol based on susceptibility of the product.

Monitoring activity is an ongoing process and samples picked for this activity are large in size. The sample number should be preferably in the range of 5 to 8 samples per location/product. The reports of these monitoring samples help the system to review quality, safety, freshness and preferences in market place. It also helps in ascertaining consumption pattern and exposures to dairy food additives and unintended contaminants and residues. The label should also specify the nature of analysis to be conducted (Qualitative/ Quantitative/Microbiological / Chemical).

1.5. Category of analysis

The category of analysis for Dairy products should be defined according to the requirement for regulatory or monitoring purpose.

- **Chemical analysis:** Required chemical tests to prove the safety of the product and nutritional tests required if product exhibits a claim.
- **Microbial analysis:** Test for Absence of pathogens and safety in microbial counts.
- **Physical analysis:** Test for extraneous matter and damaged product.
- **Sensory analysis:** Test for retention of original characteristics including flavor, texture etc. and other expected characteristics

1.6. Sampling system for physical, chemical and microbial properties

Two and three-class attributes plans are ideally suited for regulatory, port-of- entry, and other consumer oriented situations where little information is available concerning the microbiological history of the lot. The plans are independent of lot size if the lot is large in comparison to sample size. The relationship between sample size

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and lot size only becomes significant when the sample size approaches one tenth of the lot size, a situation rarely occurring in the bacteriological inspection of dairy products.

When choosing a plan one must consider: The type and seriousness of hazards implied by the microorganisms; and the conditions under which the dairy food is expected to be handled and consumed after sampling.

Table 1. Sampling points to be associated with the type of characteristic

Type of Characteristic	Type of Sampling Point
<p>Physical characteristics : Characteristics that may be expressed by two excluding situations as passed/not passed, yes/not, integer/not integer, spoiled/not spoiled (e.g. as applied to visual defects such as loss of color, miss grading, extraneous matter etc.).</p>	<p>By 'Attributes'</p>
<p>Chemical characteristics: Characteristic that may be expressed by continuous variables. They maybe normally distributed (e.g. most analytically determined compositional characteristics such as moisture content, fat content, lactose content, protein content etc...) or they may be non-normally distributed.</p>	<p>'Variables with unknown standard deviation' for normally distributed Characteristics and 'attributes' for characteristics whose distributions deviate significantly from normal</p>
<p>Microbiological properties (e.g. in the assessment of microbial spoilage, microbial hazards, irregularly occurring chemical contaminants etc.)</p>	<p>Specified sampling points to be proposed appropriate to each individual situation. Points to determine incidence rates in a population may be used.</p>



1.7. Documentation

The sampling report should include the reason for sampling, the origin of sample, the sampling method and the date and place of sampling together with any additional information like transport time and conditions. Any deviation from the specified sampling procedure to be reflected in report.

1.7.1. Documentation Form

(To keep any article of dairy product in safe custody of the vendor)

To _____

(Name and address of the vendor)

Whereas *... intended for food which is in your possession appears to me to be adulterated/misbranded:

Dairy product safety Officer _____

Area _____

Place: _____

Date: _____ // _____ // _____
 Day Month year

*Here give the name of article of food. _____

The sample described below is sent here with for analysis under ___ of ___ of section of Food Safety and Standards.

Code Number _____

Date and place of collection _____

Nature of articles submitted for analysis _____

Nature and quantity of preservative, if any, added to the sample. _____

A copy of this memo and specimen impression, of the seal used to seal the packet of sample are being sent separately by post/courier/hand delivery (strike out whichever is not applicable)

(Sd/) Dairy product Analyst Address:

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Self-check 1

Written test

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I Short Answer Questions

1. What are the purpose of sampling?(4pts)
2. What characters will be documented during sampling?(3)

Test II Fill the blank space

- _____ 1. Are picked for evaluation in case of certain concerns, issues (2pts)
- _____ 2. Is an ongoing process and samples picked for this activity are large in size(2pts).

Test III Write true if the statement is correct and false if statement is incorrect

1. During monitoring sample number should be preferably in the range of 5 to 8 samples per product. (2pts)
2. Two and three-class attributes plans are ideally suited for regulatory, port-of- entry, and other consumer oriented situations?(2p)

Note: Satisfactory rating - 12 points Unsatisfactory - below 12points

You can ask you teacher for the copy of the correct answers.

Score = _____

Rating: _____



Information Sheet 2- Determining sampling size

2.1. Commonly Used Terms and Notions

Some of the more commonly used terms in sampling are described in this section.

Lot

A lot is a definite quantity of some commodity manufactured or produced under conditions, which are presumed uniform for the purpose of these Guidelines.

A continuous series of lots is a series of lots produced, manufactured or commercialized on a continuous manner, under conditions presumed uniform. The inspection of a continuous series of lots can only be achieved at the production or processing stage.

Consignment

A consignment is a quantity of some commodity delivered at one time. It may consist either a portion of a lot, either a set of several lots. However, in the case of statistical inspection, the consignment shall be considered as a new lot for the interpretation of the results.

A representative sample is a sample in which the characteristics of the lot from which it is drawn are maintained. It is in particular the case of a simple random sample where each of the items or increments of the lot has been given the same probability of entering the sample.

Sampling

Procedure used to draw or constitute a sample. Empirical or punctual sampling procedures are sampling procedures, which are not statistical-based procedures that are used to make a decision on the inspected lot.

Item: An actual or conventional object on which a set of observations may be made, and which is drawn to form a sample.

Increment: Quantity of material drawn at one time from a larger quantity of material to form a sample.

Sampling point

Procedure which enables one to choose, or draw separate samples from a lot, in order to get the information needed, such as a decision on compliance status of the lot. More precisely, a sampling point is a scheme defining the number of items to

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collect and the number of non-conforming items required in a sample to evaluate the compliance status of a lot.

The Characteristic

A characteristic is a property, which helps to identify, or differentiate between, items within a given lot. The characteristic may be either quantitative (a specific measured amount, plan by variables) or qualitative (meets or does not meet a specification, plan by attributes).

Homogeneity

A lot is homogenous *relative to a given characteristic* if the characteristic is uniformly distributed according to a given probability law throughout the lot.

A defect (nonconformity) occurs within an item when one or more, quality characteristic does not meet its established quality specification. A defective item contains one or more defects. Lot quality may be judged in terms of the acceptable percentage of defective items or the maximum number of defects (nonconformities) per hundred items, in respect of any type of defects. Most acceptance sampling involves the evaluation of more than one quality characteristic, which may differ in importance with respect to quality and/or economic considerations.

Quantity of sample to be sent to the public analyst:- The quantity of sample of Dairy product to be sent to the public analyst / Director for analysis shall be as specified in the Table below: While defining these please bear in mind microbiological and chemical tests.

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Table 2. Quantity Recommended in FSSA

No	Type of Dairy product	Approximate quantity recommended in FSSA
1	Milk	500 ml
2	Sterilized Milk/UHT Milk	250 ml
3	Yoghurt	300 gm.
4	Cheese	200 gm.
5	Evaporated Milk/Condensed Milk	200 gm.
6	Ice-cream /Softy/Ice Candy/Ice lowly	300 gm.
7	Milk Powder /Skimmed Milk Powder	250 gm.
8	Butter/Butter Oil/Ghee/Margarine/Cream/	200 gm.

**Self-check-2****Written test**

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What is lot? (2pts)
2. Write down the sample size for different dairy products? (2pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

1. A defect (nonconformity) occurs within an item when one or more, quality characteristic does not meet its established quality specification. (2pts)
2. Homogeneity is a property, which helps to identify, or differentiate between, items within a given lot. (2pts)

Note: Satisfactory rating - 8 points Unsatisfactory - below 8 points

You can ask you teacher for the copy of the correct answers.

Score = _____

Rating: _____



Information Sheet 3- Selecting and sterilizing sampling tools and equipment

3.1. Sampling equipment

The basic tools needed are:

- an agitator,
- a dipper,
- sample containers and
- a sterilizer.

It is important that the material of the equipment used does not affect the test results. Sampling equipment should preferably be made of stainless steel. Alternatively, other suitable material of adequate strength can be used, for example adequately galvanized iron. Solder should be capable of with standing a sterilizing temperature of 180 °C. All surfaces should be smooth, free from cracks and all corners rounded.

3.1.1. Agitators

Agitators (also called plungers) for mixing milk need to be large enough to produce adequate mixing. In view of the different shapes and sizes of containers, no specific design of agitator can be recommended for all purposes, but the design should be such that damage of the inner surface of the container is avoided during mixing. For mixing liquids in buckets or cans, an agitator of the design and dimensions shown in figure 1 is suitable. The length can be adjusted to the depth of the can. An agitator of the design and dimensions shown in the figure is suitable for use for larger vessels

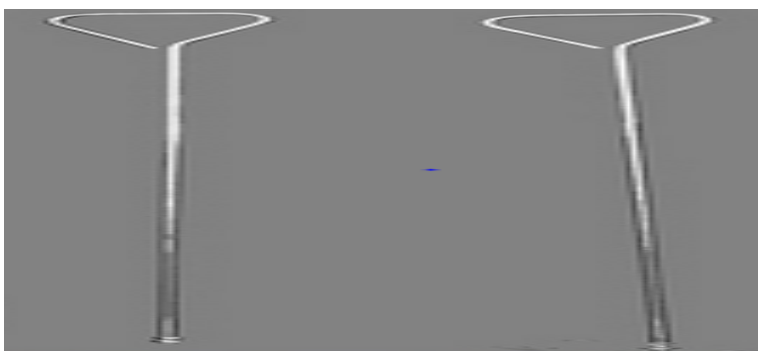


Figure 1: Agitator (plunger) for cans and buckets and large containers

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3.1.2. Dippers

A dipper of the shape and size shown in figure 2 is suitable for collecting samples. The capacity of the sample containers shall be such that they are almost completely filled by the sample taken by the dipper.



Figure 2. Dipper for taking samples

3.1.3. Sample containers

Sample containers should adequately protect the sample and not affect the test results. Appropriate materials include glass, some metals (e.g. stainless steel) and some plastics (e.g. polypropylene). The containers should preferably not be transparent, but if they are transparent they should be stored in a dark place. Containers and closures should be

- Dry,
- Clean and
- Either sterile or suitable or sterilization by one of the methods described below.

The shape and capacity of the containers depend on the particular requirements of sampling, and could be e.g. 100, 150 or 250 ml. It is desirable to avoid air space by filling the bottles to the top, leaving however sufficient space to allow for expansion of the rubber stopper. Single-service plastic containers as well as aluminum foil of adequate strength (sterile and non-sterile) and suitable plastic bags, with appropriate methods of closure, may be used.

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Figure 3: sampling containers

Containers other than plastic bags should be securely closed either by a suitable stopper or by a screw cap of metal or plastic material. If stoppers are used, they should be made from non-absorbent, odorless material. Do not use cork stoppers or caps with cork seals on containers for micro- biological examinations. Sample bottles, which are to be examined for flavor should be closed with greaseproof, non-absorbent stoppers to avoid change of odour or taste.

3.1.4. Sterilizer

This used to sterilize tools and equipment's used during sampling of a dairy product.



Figure 4. Milk Sterilizer

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3.2. Sterilizing of sampling equipment

Sampling equipment has to be clean and sterilization is required for microbiological testing. Disposable plastic equipment also needs to be sterile. Sterilization can be performed by one of the two following methods:

- A. Exposure to hot air at 170-175 °C for not less than 2 hours.
- B. Exposure to steam at 121 \pm 1 °C for not less than 20 minutes in an autoclave.

After sterilization by method A or method B, sampling equipment should be stored under sterile conditions. If, in a particular situation, sterilization by method A or method B is not possible, methods C, D or E below can be used. These methods are to be regarded as secondary methods only, and sampling equipment has to be used immediately after sterilization:

- C. Exposure to suitable flame working surfaces of the sampling equipment comes into contact with the flame.
- D. Immersion in at least 70% ethanol solution.
- E. Ignition with 96 % ethanol. (CAUTION: 96 % ethanol is hygroscopic its concentration may change over time).
- F. After sterilization by method C, D or E, sampling equipment should be cooled under sterile conditions or, in the case of method D, be rinsed with the ethanol solution before sampling.
- G. Rubber components of sampling equipment can be sterilized by immersion for at least 10 minutes in boiling water if they are used immediately. Rubber stoppers can be sterilized in an autoclave as in method B.

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Self-check-3

Written test

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. List and describe sampling equipment's?(5pts)
2. How to sterilize sampling equipment's?(5pts)

Test II: write true if the statement is correct and false if the statement is incorrect

1. Sterilizer used to sterilize tools and equipment's. (2pts)
2. Agitators or plungers are used for mixing milk.(2pts)

Note: Satisfactory rating - 14 points

Unsatisfactory - below 14 points

You can ask you teacher for the copy of the correct answers.

Score = _____

Rating: _____



Information Sheet 4- Documenting and Implementing sampling Requirement

4.1. Method of sample collection, labeling, storing, and dispatching

The first step of lab operation is collection, transportation, handling and storage of milk samples following a standard process in order to maintain the quality of milk samples that is fit for testing purpose. In the following sections, a standard process has been explained for the lab and field staff.

4.2. General requirement for sample collection

Make arrangement of all necessary materials before going to sample collection. Please take the following items for collection of milk samples.

- Clean, dry, leak-proof, sterile container (mainly plastic) with graduation/calibration on the body and polythene zip bag;
- Glass Beakers , 100 ml;
- A plunger/ dipper;



Sterile containers in polythene zip bag



Marker



Glass Beakers



Leak-proof sterile container



Face mask



Apron



A milk sampling plunger



A milk sampling dipper

- A cool box/ thermos flask to carry the sample;
- Required ice/gel packs in cool box to keep the sample cool during transportation;
- Personal protective clothing like apron, gloves, mask, etc.;
- Sticker tags, marker, note pad, mask, sanitizers and biohazard bag;
- A disposal bag for carrying disposable materials like leftover milk, gloves, mask, etc.;
- A hand sanitizer to sanitize hands of the sample collector;
- A small weighing balance to weigh if milk products (e.g. paneer, curd, etc.) are to be collected;

4.3. Information to be collected along with the sample

The following information need to be collected from the household/source at the time of sample collection:

- Type of the sample (e.g. milk/curd /cream/others)
- Species: Cow/Buffalo;
- Type of animal: Exotic/ CB/Nondescript;

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- If milk sample is collected from the cow: Quarter of udder: L (left)/R(right): F(fore)/R(rear);
- If bulk milk is collected:
 - ✓ Time of milking;
 - ✓ Weight/ volume of the sample;
 - ✓ Place of collection;
 - ✓ Date and time of collection;
 - ✓ Name and designation of the collector and Purpose of collecting the samples;
 - ✓ Name, address and thumb impression/signature of the person from whom the sample has been taken.
- All samples should be marked with a unique sample number

The above information shall be recorded against the specific sample number allocated to each sample collected and part of the information shall be supplied with the sample to the lab.

4.4. General considerations in sample collection, handling and storage

The samples should never be touched with bare hands. Gloves and mask should always be used in the process of collection. Knife/dippers/plungers, instruments used for cutting, removing and manipulating samples (e.g. paneer, sweets, etc.) should be sterilized with hot water before and after use. Sample should not be exposed to dirty materials/environment after collection and should not be mixed with other biological samples. Temperature and pH shall be recorded at the collection stage and after transporting to the laboratory. Disinfect the surface of the work area before opening the samples for measuring, packaging, etc. at the laboratory.

Sample should preferably be measured directly in the sterile container with graduation; Gloves mask and other materials in contact with the sample must be disposed properly. The stopper/cover of the container shall be securely fastened to prevent leakage of the contents in transit.

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4.5. Method of milk sample collection from milk container

In order to collect milk sample for testing purpose, following methods should be followed:

- Agitate the liquid milk thoroughly before sample is taken in order to make the contents of a milk container as homogenous as possible for obtaining a representative sample.
- Never agitate too vigorously because air bubbles, if dispersed in milk, will change its physical properties and disturb the analysis.
- Use a plunger or a dipper having a handle long enough for doing this and immediately take the sample of required volume into a sample bottle and close it.
- In order to make sure that a sample will well represent the whole contents of milk can take the half of the required sample from the lower portion and another half from upper part of the milk can.
- To take sample from a smaller milk container, turn the container upside down few times before sampling ensuring the container is closed well.
- Agitate the sample carefully again before the sample start to analysis in a laboratory.

4.6. Sampling from several containers

If milk needs to be collected from several containers, the following procedure shall be followed;

- Mix the content of each container thoroughly, take equal volume of milk from each container and pour into a small container.
- Take a sample after mixing the combined sample.

4.7. Sampling from storage tanks and rail and road milk tankers

If milk needs to be collected from a large tanker, the following procedure shall be followed. The method of sampling of milk from storage tanks and rail and road tankers is largely governed by storage/transport conditions.

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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.

4.8. Collecting milk sample directly from cow

To collect milk sample directly from a cow, the following procedure shall be followed

- Ask the owner of the cow to clean the udder and teats of the cow thoroughly with water.
- Put on the clean gloves, face mask, apron, etc. Strip two to three streams of milk from each teat in order to flush the teat canal and thereby to reduce contamination risk.
- Dry teats thoroughly with an individual cloth towel, paying close attention particularly to the teat end.



While holding the top of the teat steady, wipe the end of the teat well with an alcohol soaked cotton ball. Use as many cotton balls as necessary until the cotton ball still looks clean after using.

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Open the milk vial and immediately take the sample, making sure not to touch the inside of the tube or bottom part of the lid. Hold the milk vial about 3 inches from the teat end and fill the tube half to three-quarters full of milk. Hold the vial at a 45 degree angle to prevent dirt from falling into the vial.



Close the lid immediately and label the top with the date, cow number, and quarter sampled.



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Put the sample in cool box immediately. Note: Do not place the teat inside the vial when sampling.

4.9. Sample collection, labeling and storing

- About 50-100 ml or gm. of milk and milk products should be collected for testing purpose.
- Separate sterile container should be used for each sample.
- Immediately after collection of each sample, it should be properly labeled stating sample no., date of collect, time of collection etc.

If sample is collected for regulatory purpose (as advised by Food safety Officer), a paper slip of the size that goes round completely from the bottom to top of the container, bearing the signature of the Designated Officer and number of the sample, shall be pasted on the wrapper, the signature or thumb impression of the person from whom the sample has been taken, shall be affixed in such a manner that the paper slip and the wrapper both carry a part of this signature or the thumb impression. The outer covering of the packet shall also be marked with the same number of the sample.

- The labeled container should immediately be transferred to the cool box/thermos flask filled with ice packs.
- The collected container shall be properly secured and sealed so that no tempering is possible after collection. To ensure this, signature of the milk producer/trader/sweet maker and a witness should be taken on the sealed pack.
- All samples should be transported to the laboratory by maintaining cold chain in a cool box/thermos flask with gel/ice packs.
- After arriving the sample at the laboratory, a separate code should be assigned to each sample at the reception desk. The sample should be processed in the laboratory and results should be mentioned against that code only.
- No personal details of the owner of the sample should be supplied to the laboratory technicians who conduct the tests to avoid any potential pre-judgment by the lab technicians.

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- The sample should be stored at 4°C if milk is tested within a day or two (maximum 96 hours).
- In case of preserving the sample for longer duration, samples need to be stored at (-) 20°C.



Figure 5. Deep freezer

4.9. Packaging and dispatching of samples to other laboratories

For dispatching the samples to other laboratories, sample with their details should be put in a thermo-cool box. Adequate quantity of cool pack/gel pack should be put in the box to keep the sample cool during the time of transportation.



Fig 6. A Cool box



fig.7. Gel pack

- The outside of the thermo-cool box should be wrapped up with white paper and address of 'From' and 'to' should be clearly written on it preferably in all capital letters.

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- A certificate should also be enclosed with the box stating the nature of the materials and purpose of sending.
- Packages should be marked clearly to provide information about the contents of package and, nature of the hazard, if any.
- Sample should be sent by the mode of transportation that can deliver the sample at the quickest possible time in the destination.

If the transportation time is more, the ice/ gel pack may come to normal temperature and the sample may get spoiled. In order to avoid this, adequate no. of ice/ gel pack should be put in the thermos-cool box. There shall not be empty space inside the box as it will allow movement of samples as well as allow the gel/ice pack to get melted early. The thermo-cool box should be marked with 'Handle with care' and an 'Arrow mark' showing upside of the box, in order to guide the handlers during handling and transportation.

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**Self -cheek 4****Written test**

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. What point should be full filled during sampling?(4pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

1. Minimum time should be taken to analyses and report the findings of the disputed sample. (2pts)
2. A representative sample is essential when pathogens or toxins are sparsely distributed within the dairy food. (2pts)
3. Avoid dispatching of Samples to the Public Analyst to avoid any discrepancies in testing.(2pts)

Note: Satisfactory rating - 7 points

Unsatisfactory - below 7 points

You can ask you teacher for the copy of the correct answers.

Score = _____

Rating: _____



Information Sheet 5- Implementing safety hazards and control methods, legislations and policies and procedures

5.1. Health hazards

Similar to all other food types, milk and milk products can cause food-borne illness. The quality of milk can be affected by such factors as pathogen contamination and growth, chemical additives, environmental pollution and nutrient degradation.

5.1.1. Microbiological hazards

Microbiological hazards are a major food safety concern in the dairy sector because milk is an ideal medium for the growth of bacteria and other microbes. These can be introduced into the milk from the environment or from the dairy animals themselves.

Milk can contain harmful microorganisms such as:

- Salmonella,
- Escherichia coli O157:H7,
- Listeria monocytogenes,
- Staphylococcus aureus,
- Yersinia enterocolitica,
- Bacillus cereus,
- Clostridium botulinum,
- Mycobacterium bebies,
- Brucella abortus and
- Brucella melitensis

5.1.2. Chemical hazards

Chemical hazards can be un intentionally introduced into milk and milk products, making them unsafe and unsuitable for consumption.

Milk can be contaminated when the milking animals consume feed and/or water that contain chemicals. Other causes of contamination may be inadequate control of equipment, the environment and milk storage facilities. Chemical hazards include: detergents,

- teat disinfectants,
- dairy sanitizers,
- anti-parasitic,
- antibiotics,
- herbicides,
- pesticides and
- Fungicides.



5.1.3. Physical hazards

Apart from chemical hazards, there are also physical hazards that may influence the safety of dairy products. Physical hazards include:

- Metal parts (wire, needles etc.),
- sand/soil,
- stones,
- wood,
- plastic,
- Rubber or glass parts and hair.

They may be introduced during production of dairy products through the use of jewelry, as parts of machinery (e.g. metal parts from stirring machines or rubber from seals) or equipment, due to packaging materials or via presence in raw materials or the environment. At the farm, physical hazards may be introduced during milking (e.g. machine parts). However, in most cases, physical hazards are introduced during the further steps in the production process of dairy products.

5.2. Control measures

The majority of chemical and physical hazards are controlled by the implementation of quality assurance systems at the farm and the dairy factory. Both industry and government have to make efforts in order to ensure that food safety remains at a high level. The government should invest in knowledge developments towards future trends that may affect food safety (e.g. the application of nanoparticles or effects of climate change on food safety). Furthermore, a proper food safety control as performed by the NVWA is essential. However, the NVWA cannot control all food safety hazards at the different stages of the dairy chain and, therefore, inspections are organized on a risk basis, focusing on the most important food safety hazards at the main steps of the dairy chain.

- The use of **food safety** and **quality assurance** in farms and plants is very important to reduce chemical and physical hazards in milk and dairy products.
- A regulatory law implementation in milk and dairy industries and long term planning is required to do milk safety.
- In addition, there are other items such as training of personnel or current good manufacturing practices and monitoring.

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- Good manufacturing practices and monitoring can do by regular examination with economic penalties about chemical residues.
- It is useful to reduce chemical contaminants in milk and dairy products from a regulatory point.
- Monitoring can accompany by regular reevaluation of the acceptable levels must continue, but with the realization that some residues will probably always be found in very low quantities and they are considered to be unavoidable contaminants.
- List all potential hazards associated with each step; conduct a hazard analysis; and identify any measures to control identified hazards.

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Self-cheek- 5	Written test
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Directions: Answer the questions below.

1. List the different types of hazards and there safety measures?(5pts)

Note: Satisfactory rating - 5 points

Unsatisfactory - below 5 points

You can ask you teacher for the copy of the correct answers.

Score = _____

Rating: _____



Operation Sheet 1– sampling for milk and milk products from Individual Containers

Sampling of milk

Objective

- To know how representative samples can be taken.

Materials: PPE, sampling kit, recording book, parker, sealing material.

Procedure:

Step 1. Mix thoroughly the milk from one container to another container for five times to ensure uniformity of milk.

Step 2. Do not allow the milk to stand for longer than five minutes after mixing and take required quantity of milk with the help of a dipper.

Sampling from Several Containers:

step 1. Mix the milk thoroughly with the plunger.

step 2. Take proportionate quantity of milk in a separate vessel.

step 3. Repeat this procedure for all cans.

step 4. Mix the milk from separate vessels in one from which proportionate quantity of milk samples from different cans are taken.

step 5. Take final sample from vessel with the help of a dipper.

Sampling of Cold Canned Milk:

Step 1. In cold can milk there is formation of cream line; break the cream line before sampling.

Step 2. Dump the cans into weight vat/tipping tank. Record the temperature of the milk.

Step 3. Adjust the temperature of the milk to 90-104⁰F.

Step 4. Stir, the milk thoroughly with plunger or agitator.

Step 5. Take small quantity of sample at three or more different places from the vat.

Sampling of Partially Churned Milk and Cream:

Step 1. Heat the milk to a temperature of 90-104⁰F.

Step 2. Stir, the milk thoroughly and take representative sample immediately.

Sample of Cream:

Step 1. Warm the cream to 122⁰F

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Step 2. Mix it thoroughly and

Step 3. take a representative sample.

Sampling of Butter:

Step 1. Remove three or four cores with a sample Trier from various parts of the products mass.

Step 2. Core should be extending from exterior to the center.

Step 3. Keep the combined cores in wide and necked bottle for analysis.

Composite Sample:

Step 1. properly prepare represents two or more lots of milk.

Step 2. The sample must be taken in proportion to the amount of milk in each lot.

Step 3. Such milk is placed in a properly labeled bottle and tested after a week or two.

Step 4. The results obtained would give an average figure for total amount of milk received during the period.

Step 5. The purpose of this is to reduce the number of analysis and loss of chemicals, labor etc.

Step 6. The volume of composites sample should not be less than 175 ml.

Step 7. The milk is preserved by adding preservatives to prevent souring since composite samples are kept for longer period.

Step 8. Formalin 36% is added at 0.1 ml. for 25 ml. of milk.

Step 9. Dichromate of potash: required for metal container 6 – 8 grains for ½ liter milk.

Step10. Label the sample properly.

Step11. During transportation, sample should not be exposed to sunlight, or not to be exposed to near volatile odour as milk picks them up immediately.

Step12. Use an air tight container, it should have 100 – 250 ml. capacity and should be rubber stopper.

Step13. Keep the sample in a cool place at 45 – 60⁰F.

Step 14. In composite milk sample each time when milk is added, mix the sample thoroughly.

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LAP TEST	Performance Test
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Name.....ID.....Date.....Time
started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within 2 hour. The project is expected from each student to do it.

Task-1 Perform sampling practices for milk.

Task-2 Perform sampling practices for milk product.



LG #33 LO 2- Monitor chemistry in dairy products processing

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Recording acidity profile
- Carrying out tests of Salt, Moisture and Fat levels
- Analyzing whey content for fat
- Reviewing and Establishing safe work procedures for processes

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Record acidity profile
- Carry out tests of Salt, Moisture and Fat levels
- Analyze whey content for fat
- Review and Establishing safe work procedures for processes

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.



Information Sheet 1- Recording acidity profile

1.1. Determination of acidity profile in milk

The titratable acidity test is employed to ascertain if milk is of such a high acidity so as to reduce its keeping quality and heat stability. Generally the acidity of milk means the total acidity (Natural + developed) or titratable acidity. It is determined by titrating a known volume of milk with a standard alkali. Determination of titratable acidity of milk as Lactic Acid can be done by two methods as stated below:

- I. Conventional method
- II. By using paper strip test with color comparator

1.1.1. Conventional method

Apparatus and materials required

- 100 ml conical flask
- Distilled water
- Phenolphthalein indicator
- N/10 NaOH
- Milk sample Procedure Materials

Procedure

- Take 10 ml milk in 100 ml conical flask, add 10 ml distilled water
- Add 1ml phenolphthalein indicator and titrate against N/10NaOH till a faint pink color appears
- Calculate the acidity % as volume of NaOH used \times 0.09
- Calculation Titratable acidity % (as lactic acid) = $\frac{9V_1N}{V_2}$

Where

V₁ = Volume in ml of the standard sodium hydroxide required for titration,

N = Normality of the standard sodium hydroxide solution, and

V₂ = Volume in ml of milk taken for the test

1.1.2. By using paper strip test with color comparator

These strips are commercially available in the market.

Procedure:

- As per the instruction given within the kit Advantage of using Milk Adulterant Kit :

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- It gives an instant result.
- Easy to perform.

1.2. Determination of Acidity in Yoghurt

Yogurt is a fermentation product made from milk and bacteria. Bacteria may sound gross, but not all bacteria are bad. The bacteria in yogurt are good bacteria. These bacteria are generally:

- *Lactobacillus bulgaricus*,
- *Streptococcus thermophiles* and
- *Lactobacillus acidophilus*.

They work together to convert the lactose sugar in milk to produce lactic acid and flavors. This lactic acid in turn causes the proteins in the milk to coagulate to form a thick gel. The production of lactic acid is essential for texture, taste and shelf life, as acidic environments inhibit pathogen growth.

Titrateable acidity is an approximation of the total acidity in a substance. It determines how much of a base (NaOH) is required to neutralize an acid. The base, also known as the titrant, is of known concentration.

A color indicator will be used to determine the end point of the acid-base reaction. The indicator is phenolphthalein. Phenolphthalein is colorless in acid and turns pink in basic solutions. Therefore, when the yogurt turns pink, it means that all of the acid has been neutralized. The following formula used to calculate the total acidity.

$$\text{Titrateable acidity} = \frac{\text{nxvolume of titrant} \times N \times 90}{\text{weight of sample} \times 1000} \times 100$$

Where, N = normality of titrant; 90= Equivalent weight for lactic acid

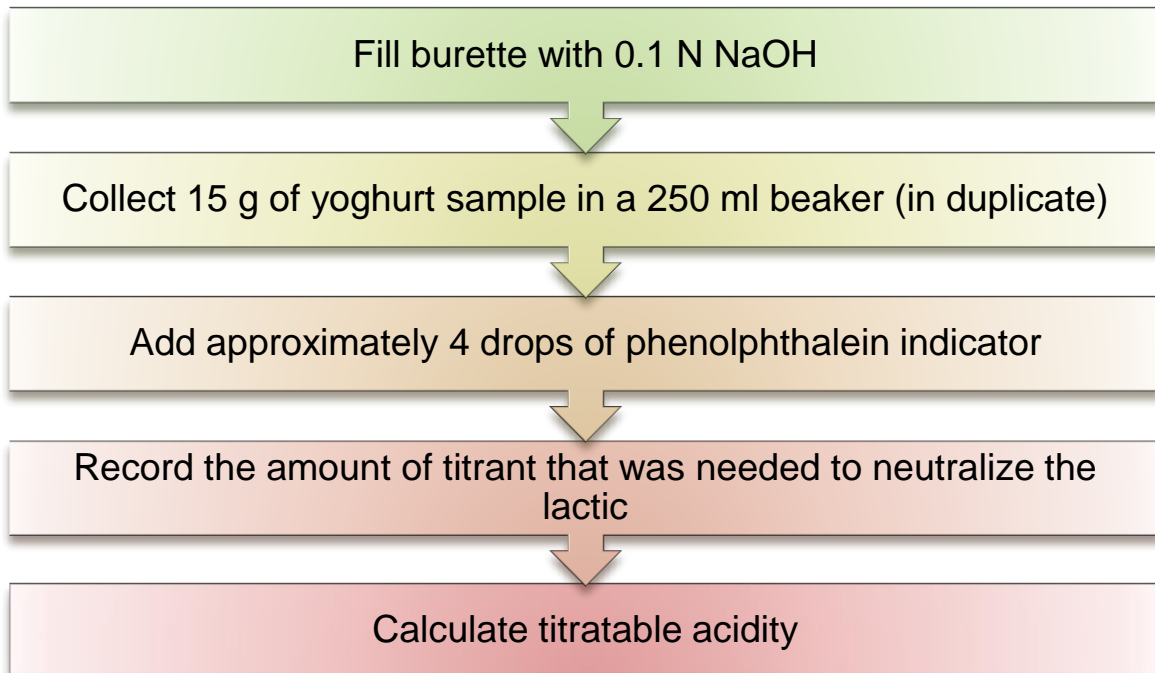
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1.2.1. Materials and Equipment

Three containers of plain yogurt representing different commercial brands 1 N sodium hydroxide, Phenolphthalein indicator, Scale (Capacity = 0-100 g), Burette, Magnetic stir plate, Magnetic stir bar are required.

Procedure:



1.3. Determination of Titratable Acidity in Dried Milk

The method is based on the titration of the sample with sodium hydroxide to phenolphthalein end point and by comparing the color with color obtained by mixing rosaniline acetate or cobalt sulphate to a known volume of milk sample.

Procedure

- 0.1N Sodium hydroxide solution is needed as reagent
- Rosaniline acetate solution: Dissolve 0.12 g rosaniline acetate in 95% ethyl alcohol containing 0.5 ml of glacial acetic acid and diluted to 100 ml (Store in dark). To prepare working standard, dilute 1 ml to 500 ml with 95% ethyl alcohol and water in the ratio of 1:1.
- Phenolphthalein solution: To 1 g of phenolphthalein dissolved in 100 ml of 95% ethyl alcohol, add 0.1N sodium hydroxide till a faint pink color is obtained and dilute to 200 ml with distilled water.



- Cobalt sulphate solution: Dissolve 1.5 g of cobalt sulphate in water and dilute to 100ml.
- Porcelain dishes 100 ml.
- Burette 5 ml.
- Glass rods for stirring.

1.4. Determination of Titratable Acidity in Cheese

Titrate acidity (TA) measures all titratable H⁺ ions up to the phenolphthalein end point (pH 8.5) and, therefore, varies with changes in milk composition and properties. During cheese manufacture, the pH gives a true indication of acid development during the entire process so that the optimum pH at each step is independent of other variables such as milk protein content. However, the optimum TA at each step in cheese making will vary with initial milk composition and the type of standardization procedure used.

A good practical illustration of the difference between TA and pH is the effect of cutting. Up to the time of cutting, TA of the milk increases with the development of acidity by the culture. After cutting the TA of the whey is much lower. This does not mean that acid development stopped. It simply means that titratable H⁺ ions associated with the milk proteins are no longer present in the whey. This leads to the concept of buffer capacity, which is an important principle in cheese making. The effect of protein removal on the TA of whey is related to the ability of protein to 'buffer' the milk against changes in pH. That same buffer property is the reason it helps to take acidic medication, like aspirin, with milk.

Materials and Reagents

- An acidometer equipped with a burette graduated in tenths of a ml up to 10 ml, and some means of filling the same without undue exposure of the solution to the carbon dioxide of the atmosphere.
- N/10 sodium hydroxide solution.
- A dropping bottle containing a 1% alcoholic phenolphthalein solution.
- White cup, glass stirring rod, 17.6 ml pipette (or 8.8 or 9.0 ml pipette)
- For cream, Torsion balance and 9 g weight.

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Procedure

- Mix sample thoroughly by pouring it from one container to another. The temperature of the sample should be near 20C.
- Pipette 17.6 ml of milk or cream into a white cup. Note: 8.8 ml pipettes may also be used but are no longer as readily available as 17.6 ml pipettes. Readily available 9 ml pipettes are also used but require application of a correction factor to the final result.
- Add six drops of phenolphthalein indicator solution to milk, 10 drops if the product is cream.
- Titrate the sample with the N/10 sodium hydroxide solution (0.1 Normal NaOH) while stirring the sample with the glass rod. Look for the appearance of a faint pink colour which signals the endpoint. Add another drop or half a drop of NaOH if the pink colour does not persist for 30 s.
- Record the number of ml of NaOH used to reach the endpoint. This value is called the 'titre'. Titratable acidity reported as percent lactic acid is dependent on the volume of sample.
 - ✓ For the 8.8 ml pipette, % Lactic acid = titre
 - ✓ For the 17.6 ml pipette, % Lactic acid = 0.5 x titre
 - ✓ For the 9.0 ml pipette, % Lactic acid = 0.98 x titre.
 - ✓ Note that there is practically no lactic acid in fresh milk, but it is a North American convention to report TA in terms of % lactic acid.

Table 3. Recommended Acidity profile of milk and milk products

Dairy product	Acidity profile in %
Milk	0.16-0.18%
cheese	0.2%
Sweet whey	<0.2%
Skimmed milk	≤18%
cream	≤ 0.2%
Casine	≤ 0.27
Ghee	≤ 0.3%
Yoghurt	4.6 PH



Self-check 1	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Determine the procedures of determining acidity of milk? (3pts)
2. List materials used to test acidity in yoghurt? (3pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

1. Bacteria that normally grow in raw milk produce ferment lactose and produce lactic acid.
(2pts)
2. Titration of the acidity in milk determines the acidity of milk and milk products.
(2pts)

Note: Satisfactory rating - 9 points Unsatisfactory - below 9 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____

Information Sheet 2- Carrying out tests of Salt, Moisture and Fat levels

2.1. Determination of Fat in Milk

Fat is the most important component of milk. It provides more energy than the energy provided by carbohydrate (lactose) and proteins taken together. It imparts soft texture and creamy taste to milk products. It is the source of essential fatty acids and carrier for fat-soluble vitamins. Due to these reasons the producers and/ or sellers of milk and milk products are paid for their product on the fat basis. Therefore, determination of fat in milk and its products is an important exercise.

Fat in milk is tested by two methods: Conventional method by using Gerber centrifuge and by using a Digital milk analyzer

A. Gerber Method

Apparatus and reagents required

- Sulphuric acid (Specific Gravity 1.807 – 1.812 g/ml at 270C, colorless).
- Amyl alcohol (Specific Gravity 0.810 to 0.812) conforming to grade 1 of IS: 360:1964.
- Butyrometer 10% Scale
- Stoppers and shaker stands for Butyrometer made from a suitable grade of rubber or plastics.
- 10 ml Acid pipette for sulphuric acid (with rubber suction device).
- 10.75 ml pipette for milk.
- 1 ml automatic measure for amyl alcohol
- Centrifuge, electric or hand driven (1400± 70 RPM)
- Water bath at 65 + 20C Method



Fig 8. A Butyrometer

Procedure

- Transfer 10 ml of sulphuric acid into a butyrometer using a 10 ml acid pipette.
- Fill the 10.75 ml pipette with milk and deliver the sample into butyrometer.



- Add 1 ml of amyl alcohol using a 1 ml pipette and close.
- Shake the butyrometer in the shaker stand until no white particles are seen and invert it a few times.
- Put the butyrometer in the water bath for 5 min.
- Take it out and dry with a cloth, put it in the centrifuge, placing two butyrometers diametrically
- opposite, centrifuge at maximum speed for 4 minutes.
- Transfer the butyrometers, stoppers downwards into water bath for 3-10 minutes.
- Bring lower end of fat column on to a main graduation mark by slightly withdrawing stopper

Points to be considered:

- The colour of the fat should be straw yellow;
- The ends of the fat column should be clear and sharply defined;
- The fat column should be free from specks and sediment;
- The water just below the fat column should be perfectly clear;

The fat should be within the graduation. Interpretation Note down the upper and lower scale readings corresponding to the lowest point of fat meniscus and surface of separation of fat and acid. The difference between the two readings gives the percentage by mass of fat in milk. The reading has to be done quickly before the milk cools.

Note: The Butyrometer should be emptied into a special container for the very corrosive acid milk liquid, and the Butyrometer should be washed in warm water and dried before the next use. Fat testing is often carried out on composite or random samples in order to reduce time and costs.

B. Digital milk analyzer

Milk fat is also measured by using a Digital milk analyzer. Put the milk sample in a container that fits into the Digital milk analyzer and place it in the machine. The analyzer will give instant results for fat percentage. Digital milk analyzer is also used for assessing SNF%, salt, sugar, added water, etc. in milk. Advantage of Digital milk analyzer:

- Gives instantaneous result

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- A printed test results can be obtained in some machines
- Recurring cost is minimal
- Easy to perform the test Disadvantage of Digital milk analyzer:
- Needs to buy a milk analyzer involved in testing.



Fig 9. A Digital milk analyzer

4.3. Determination of Salt in Milk

Salt or sugar is used to mask extraneous water added to milk or to elevate total solids in milk. So it is important to detect presence of salt in milk. Presence of salt in milk can be detected by two methods as stated below,

- A. Conventional method
- B. By using Milk Adulterants Kit

4.3.1. Conventional method

Apparatus and reagents required;

- Test tubes
- 5% potassium chromate
- 0.1N silver nitrate
- Milk sample

Procedure

- Take 2.0 ml of milk in a test tube



- Add 1.0 ml of 5% potassium chromate to the milk
- Add 2.0 ml of 0.1N silver nitrate to the test tube

Inference Appearance of red precipitate indicates the absence of dissolved chloride in milk Appearance of yellow colour indicates presence of dissolved chloride

4.3.2. By using Milk adulterants kit

These kits are commercially available in the market Procedure as instructed by the manufacturer. Advantage of using Milk adulterants kit:

- It gives an instant result
- Easy to perform

4.4. Determination of Moisture in Cream Powder

The sample is dried to constant weight at 102°C and the loss in weight reported as moisture. Moisture %by mass= $\frac{100(m_1-m_2)}{M_1-m}$

$$M_1-m$$

Where

M = mass in g, of the empty dish;

M₁ = initial mass in g, of the dish and lid with the material taken for analysis; M₂ = final mass in g, of the dish and lid with the material after drying.

The maximum deviation between duplicate determinations should not exceed 0.06% by mass of moisture.

4.5. Determination of Fat Content in Cream Powder

Quickly weigh to the nearest mg about 1 g well mixed sample into a small beaker. Add 1 ml water and rub to smooth paste. Add 9 ml additional water and 1 – 1.25 ml NH₄OH and warm on steam bath. Transfer to fat extraction flask or tube. Cool and extract fat by Rose Gottlieb method after adding 10 ml of alcohol.

4.6. Determination of Fat in Ice-Cream (Rose-Gottlieb Method)

Accurately weigh 4-5 g of the thoroughly mixed sample directly into fat extraction flask or Mojonnier tube, using free flowing pipette, dilute with water to approximately 10 ml, working sample into lower chamber and mix by shaking. Add 2 ml ammonia; mix thoroughly, heat in water bath for 20 min at 60°C with

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occasional shaking, cool then (beginning "Add 10 ml alcohol and mix well"). Identify the clear extracted fat to confirm whether it is dairy fat or not by checking refractive index at 40°C.

4.7. Determination of Fat and Curd (Milk Solids Not Fat) In Butter

Fat in butter may be determined using direct or indirect methods. Indirect (that is, fat-by-difference) methods analyses the non-fat portion of the butter and subtract this from 100/ to calculate the fat content, whereas direct methods measure fat directly. Essentially two different groups of methods have been employed to determine fat by difference. One group of methods is based on the principle of physically separating the total non-fat portion from the fat followed by the volumetric determination of the total non-fat portion.

The other, gravimetric, group of methods separately determines the moisture and the solids-not-fat (SNF) portions of the butter. The moisture may be removed by placing a dish containing the butter in an oven, on a hot plate or above a non-sooting flame. The weight loss of the butter through a particular heating process is defined as moisture. The fat is then removed from the dried butter using a suitable solvent and the retained SNF portion.

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Self check- 2	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Short Answer Questions

1. Determine the fat level of milk, milk powder, cheese and butter. (5pts)
2. Determine the moisture content of milk powder, cheese and butter. (5pts)
3. Determine the fat content of milk, milk powder, cheese and butter(5pts)
4. Determine the salt content of milk, milk powder, cheese and butter(5pts)

Note: Satisfactory rating - 20points Unsatisfactory - below 20 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information Sheet 3- Analyzing whey fat content

3.1. Analyzing Whey Content for Fat

The whey content affected by the following variables or conditions. Whey content is affected by curd particle size cutting time, cutting speed, and subsequent agitation such as:

- Short cutting times and low cutting speed result in small particle size at draining and larger losses of whey.
- With increasing cutting time (more total revolutions), curd particle size at draining reaches a maximum which corresponds to a maximum in fat recovery.
- Further increased cutting time causes decreased curd size at draining with little effect on fat recovery.

3.1.1. Healing

Curd should be agitated gently or not at all after cutting to prevent formation of fines. The exterior of the freshly cut curd is fragile so some time is needed for the edges to close up (heal) and prevent the loss of fat and protein to the whey.

3.1.2. An index of cutting quality

The loss of fines is best monitored by accurate analysis of whey fat content. Whey fat for Cheddar types should be $<0.3\%$; Efficient operations may achieve levels near 0.2% .

3.1.3. Cooking

The combination of heat and the developing acidity (decreasing pH) causes syneresis with resulting expulsion of

- Moisture
- Lactose
- Acid
- Soluble minerals and salts, and whey proteins. It is important to follow the cooking schedule, closely.

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Cooking too quickly causes the curd to shatter more easily and forms a tough exterior on the curd particles which prevents moisture release and hinders development of a smooth texture during pressing.

3.1.4. Washing

Lactose content can be adjusted by moisture removal (syneresis), fermentation, or leaching with water. By leaching lactose with water it is possible to make a high moisture cheese (such as brine brick or Muenster) and still achieve a final pH of about 5.0 - 5.2. The temperature of the wash water will determine the moisture content of the curd. Sometimes relatively hot water (eg., Gouda) is used to dry the curd and develop its texture.

Traditionally washing was accomplished by removing Omega to 2/3 of the whey and replacing it with water and agitating for about 15 min. This process results in the dilution of large amounts of whey which must be re concentrated or dumped. It also creates problems where curd tables have less capacity than setting vats. The solution is to remove more whey and add less water.

3.1.5. Curd Handling

Most brine or surface salted varieties are dipped directly into the forms or pressed under the whey. In the absence of salt, the curd is fused to form a smooth, plastic mass. The hoops are turned at regular intervals to promote uniform drainage, symmetrical shape, and a smooth finish.

Some varieties such as Gouda and Swiss are pressed under the whey before draining. This encourages formation of smooth texture and prevents incorporation of mechanical openings in the cheese due to trapped air or pockets of whey.

3.2. General information about whey

- Fat: around 1 %
- Protein: 13 – 14 %
- Lactose (monohydrate): 72 – 75 %
- Nitrate (FIA): 140 – 180 mg/kg

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3.2. Determination whey content for Fat Content, (Rose Gottlieb), IDF 9C:1987

after homogenization about 1.5 g of dried whey (nearest to 1 mg) transferred to fat-extraction flask (Mojonnier type):

1. Prepare a blank test, instead of dried whey take 10 ml water
2. Prepare fat-collecting vessels: drying of vessels, add a few boiling aids, heating at $102 + 2^{\circ}\text{C}$ for 1 h, cooling down and weigh to the nearest of 0.1 mg.
3. Add 10 ml water at $65 + 5^{\circ}\text{C}$ to wash the test portion into the small bulb of the flasks until the product is completely dispersed.
4. Add 2 ml 25% ammonia, mixing
5. Heat the flask at $65 + 5^{\circ}\text{C}$ in the water bath, occasionally shaking.
6. Cooling down to room temperature
7. Add 10 ml EtOH, at least 94% (v/v), mix gently
8. Add two drops of Congo red solution (1 g , dilution to 100 ml)
9. Add 25 ml diethyl ether, shaking vigorously avoiding emulsions
10. Transfer organic layer into fat-collecting vessel by decantation
11. Add 25 ml light petroleum (boiling point $30-60^{\circ}\text{C}$)
12. Shaking gently and then centrifuge the flask (500– 600 rpm)
13. Allow the closed flask to stand for at least 30 min (separation of the organic supernatant layer has to be clear and separated from the aqueous layer (if necessary cool the flask
14. Rinsing of organic layer to fat-collecting vessel, adding gently some water to raise the aqueous layer and transfer again organic layer into fat-collecting vessel by decantation
15. Rinse the outside of the extraction flask with some mixed solvent, collecting the rinsing's in the fat-collecting vessel
16. If desired, solvent parts may be removed from the vessel by evaporation etc.
17. Add 5 ml ethanol to the rests in extraction flask
18. Carry out a second extraction by repeating steps 10 to 16
19. Carry out a third extraction by repeating steps 10 to 16
20. Remove solvents from fat-collecting vessel as completely as possible.
21. Heat fat-collecting vessel for 1 hour at $102 + 2^{\circ}\text{C}$, allow to cool
22. Weigh to the nearest of 0.1 mg. 24. Repeat the heating for checking removal of solvents

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Self-check 3	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Short Answer Questions

- 1. What are the factors determining whey content of fat?(5pts)
- 2. What the effect of cooking on the content of whey? (2pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

- 1. The combination of heat and the developing acidity (decreasing pH) causes syneresis with resulting expulsion of whey proteins. (2pts)
- 2. The loss of fines is best monitored by accurate analysis of whey fat content. (2pts)

Note: Satisfactory rating - 9 points Unsatisfactory - below 9 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet 4. Reviewing and establishing safe work procedures for processes

4.1. Dairy processing stages

The raw milk in the milk container truck, having passed the preliminary analytical tests, proceeds to whole milk intake bays and the milk hoses are connected up by the driver. The milk is pumped into bulk storage tanks called milk silos (capacity can be up to 300,000 l, plus). The driver enters the trucks identification number on the pump's control panel or uses a key fob (a passive wireless electronic device that usually uses radio frequency ID technology) to start pumping into the whole milk silos.

Unloaded milk is cooled automatically to 4–6°C with a heat plate exchanger (HPE) while pumped into the silo. The offload time and setup time taken to couple and decouple the milk intake hoses are areas where processing monitoring can be implemented. The pumping time can be variable, indicating performance specific to each pump and the flow rate represents a reasonable performance indicator. Other significant factors that can influence pumping time include:

- the volume of milk in the receiving silo,
- the number of bends and valves in each pipeline and
- The associated back pressure variations.

At milk offload, process optimization can be achieved by ensuring pumps are working effectively, efficiently, and planning truck supply due to intelligent time slot management.

4.2. Microbiological quality analyses

Each step along the milk processing train can be contaminating by the air and the water, used in the milk processing stages. Hygiene control at all stages, including hygienic design of the manufacturing equipment, is critically important.

4.2.1. Microbial quality analysis at farm level

The microbial quality of milk starts a farm level. Milk is sterile at secretion in the udder but is colonized by bacteria before it leaves the udder. The temperature of milk

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expelled from the udder is approximately 35°C; to prevent microbial growth, rapid cooling, and storage to 4°C is necessary.

The dairy farmer has the responsibility of managing and maintaining a clean and hygienic milking parlor with a good milking and storage routine. The farmer can detect early signs of mastitis infection by using a somatic cell count (SSC) test. Low levels of SCC (<200,000/ml) are wanted to guarantee good extraction of protein from milk. High levels of SCC also reduce other levels of milk constituent including lactose. The California Mastitis Test (CMT) offers a quick and easy on-farm test; the test does not provide a specific SCC, but will give a positive result once a cow's SCC goes over 400,000 cells/mL.

The addition of the CMT solution to milk samples with a high number of leukocytes/white blood cells causes the solution to become mucous like. This reaction is caused by the release of DNA from somatic cells, which are now higher due to the immune response of the cow to infections. Mastitis is caused by the microorganism *Staphylococcus aureus*. CMT test are available commercially from many companies.

The milk tanker driver can perform a few tests at the farm, but this is not often practical. The collector will also take a sample of raw milk and label it with a bar code identifier, to be brought back to the dairy processing plant. Composite samples are taken for the detection of inhibitory substances (e.g., antibiotics, antiseptics) to be tested later at the processing plant and if positive the individual suppliers samples are then analyzed.

4.2.2. Microbial quality testing at milk intake

At the milk intake point, the milk is tested before acceptance into the processing train to know about the microbial load. Milk having more than the required standard should be rejected to maintain the safety. The milk density is another rapid test to determine adulteration of the milk and an indication for the deviations from the normal milk composition, for example, if it has been watered down or skimmed. In this test, a dipping lacto dense meter combined with a thermometer is used (Gerber instruments; Brouwland instruments), lactometers/milk hydrometers are calibrated in either grams per milliliter (g/cm³), degrees specific gravity (SG), or Degrees Quevenne. 1° Quevenne = 0.001°SG. Density ranges for standard milk are between 1.026 and

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1.034 g/cm³. The adding of 10% water to milk will end up decreasing milk density by ~0.003 g/cm³.

4.3. Microorganisms and milk

A wide variety of bacteria grow and survive in milk, including problematic spore-forming bacteria and pathogens such as non-typhoid *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* are also found.

The common bacteria in milk are lactic acid bacteria (LAB), which can produce enough acid to reduce the pH of milk, and cause the coagulation of proteins, thus fermenting the milk. The density test as previously described should be introduced at milk intake, as it can determine the degree of LAB growth. LAB can be divided into rods (*Lactobacillus* and *Carnobacterium*) and cocci (all other genera).

Psychotropic microorganisms are also present up to 80% in fresh collected milk, they are able to grow quickly below 7°C, and some contain heat-stable enzymes, which cause spoilage, including many Gram-negative bacteria, such as *Pseudomonas fluoresces*, *Pseudomonas frag*, *Pseudomonas putida*, *Achromobacter*.

Thermoduric bacteria can survive pasteurization. They do this by forming spores, which can then carry over into the final product. This can cause quality defects in milk products such as decreasing the shelf life of pasteurized milk. They are represented mainly by Gram-positive bacteria, e.g., *Bacillus* and *Clostridium* spp., and the non-spore-forming genera, e.g., *Micrococcus*, *Streptococcus*, and *Carnobacterium*.

Levels of greater than 1000 cfu/ml are normally the result of poor cow hygiene and milking equipment (particularly in the case of ineffective hot wash routines). Potential sources of thermoduric bacteria include silage, faces, animal bedding, and soil [84].

Thermophilic bacteria grow in milk held at raised temperatures (55°C or higher), including pasteurization, 62.8°C, they include the *Bacillus* spp. Thermophilic bacteria are monitored by standard plate count methods with incubation at 55°C. However,

The total viable count (TVC), or total bacterial count (TBC), is used to indicate the overall level of microorganism in milk; *E. coli* and coliforms to indicate any fecal contamination; and *Pseudomonas* spp., to indicate any non-fecal contamination. The ruling indicates that TBC in raw milk should be less than 100,000 cfu/ml; however, a

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TBC of less than 15,000 cfu/ml is desired. A standard to aim for is <1000 cfu/ml as milk leaves the udder; <3000 cfu/ml as milk leaves the milking machine; and <5000 cfu/ml in the bulk tank. Further contamination takes place during storage and preprocessing activities.

4.4. Separation, clarification, and centrifugation

Different milk processing plants have their own process trains. In many cases, milk must be clarified on reception at the dairy, to remove particles of dirt such as: sand, soil, dust, and precipitated protein,

Which will protect downstream processing equipment In addition; removal of bacteria, spores, and somatic cells from milk can be achieved with centrifugation and microfiltration techniques.

Somatic cells such as leucocytes are removed, which will reduce the presence of Listeria trapped inside the leucocyte.

- Reduction in the microbial load at this point can decrease the burden of biofilms, which leads to more efficient work.
- Milk bacterial clarification also avoids problems during cheese aging,
- And improves shelf life and organoleptic properties of the dairy products.

A clarifier is a type of centrifugal separator, but clarifiers and milk separators serve slightly different duties. All centrifuges can act as clarifiers; however, in general, only centrifuges with a high hydraulic capacity are used in this way. The clarifier can function with either cold (below 8°C) or hot milk (50–60°C).

The main use for centrifuges in dairy processing plants is hot milk separation. The aim is to separate the globular milk fat from the serum, the skim milk. This process is known as skimming. This process is generally combined into the pasteurization line and joined with an in-line fat standardization system for both milk and cream.

Separation normally takes place at 122–140°F (50–60°C). The fat content of the cream discharged from the separator can be controlled to a level of between 20 and 70%. The terminology for separation in the dairy industry includes continuous centrifugal separation of solid particles (Clarifier), separation of cream (Separator), or separation of bacteria (Bactofuge).

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The microbial quality of milk powders is highly significant and it is possible at this early phase of processing to remove 99.9% of the spore-forming bacteria by either bacto-fugation or microfiltration preceding heat treatment.

Standardization of milk is the alteration of fat and solids-not-fat (SNF) levels, i.e., raising or lowering of these levels. This is regularly carried out for the consumer market milk supply and in the production of other milk products including: condensed milk, milk powder, ice cream and cheese, etc. Standardization is typically carried out to create a uniform milk fat content in the final dairy product.

4.5. Pasteurization

Pasteurization was originally introduced to control *Mycobacterium bebies*, which causes tuberculosis (TB), which is no longer problematic as cows are tested for TB annually and removed from herds if they test positive for the disease. The TB bacillus is a highly heat resistant microorganism; however, *Coxiella brunet*, the cause of Q fever in humans, required pasteurization of 161°F (71.7°C) for 15 s, and is the current official standard for milk pasteurization, the standard vat pasteurization is 63°C (145°F) for 30 min. However, heat processing can result in the loss of subtle aroma and flavors components, loss of vitamins and natural antioxidants, the loss of texture and freshness, and the denaturation of proteins.

4.6. Verifying the process

The Pas Lite test is an internationally accepted method used by dairies and food manufacturers to verify process for many types of dairy products. The Pas lite test verifies the completeness of milk pasteurization by detecting alkaline phosphatase, a natural enzyme in milk that is destroyed by the heat and hold time of pasteurization.

When a dairy sample is mixed with Pas Lite reagents and incubated, the resulting solution emits light in an amount directly proportional to the phosphatase enzyme present. The Charm nova LUM ATP detection system is used to measure the light emitted and converts light readings to enzyme units.

Phosphatase readings greater than 350 mu/L indicate product pasteurization issues requirements. The Pas Lite test detection limit for liquid dairy products is 20 mill units per liter (mU/L) phosphatase (~0.002% raw milk). This is much lower than the 350 mU/L level (0.1% raw milk) mandated by nearly all public health agencies.

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4.7. Intervention measures

Intervention measures for processing dairy food safety during processing can be secured by implementing basic requirements and a HACCP-plan or hygiene codes. Basic requirements are described in:

- Good Practices, like GMP (Good Manufacturing Practice) and
- GHP (Good Hygienic Practice).

GMP is the most common good practice. It consists of fundamental principles, procedures and means needed to design the basic environmental and operating conditions for food production. Guidelines are prescribed on aspects like buildings and facilities, personnel, equipment, production and process control.

GHP is a good practice code specifically focused on hygiene. The guidelines of GHP describe hygienic aspects, like:

- cleaning and disinfection,
- health and hygiene of personnel,
- Pest control and training.

Although GHP primarily focuses on microbial hazards, it also describes how to prevent physical and chemical hazards entering the food product. For example,

- detergents and disinfectants need to be stored properly,
- separate from food products,
- And food products need to be covered to prevent entrance of chemical and physical hazards.

The milk processing chain demands accurate and quality products from farm to plate and for all of its products, e.g., fluid milk, milk powders, etc. It must start with the raw material at farm level including; dairy herd improvement testing, to payment parameters, and quality control of the raw milk.

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Self-check -4	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. How can secured Intervention measures for processing dairy food safety during processing. (2pts)
2. write the difference between GHP and GMP.(2pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

1. GHP is a good practice code specifically focused on hygiene (2pts)
2. Small food producing companies work according to HACCP systems.(2pts)

Note: Satisfactory rating - 8 points Unsatisfactory - below 8 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Operation sheet 1 determination of acidity in milk

Objective; to understand the sharpness of milk

Procedure

Step1. Take 9 ml milk in the flask.

Step2. Phenolphthalein (one ml) is transferred to the milk in the flask

Step3. Add sodium hydroxide (0.1 N) under continuous mixing from the buret until development of faint pink color.

Step4. Amount of sodium hydroxide solution in ml is noted and divided by 10 expresses the percentage of lactic acid



Operation sheet 2- Determination of Fat in Casein/Caseinates

Objective: to determine the fat content of Casein

Procedure

- Step1. Mix the test sample and weigh immediately to the nearest 1 mg, directly or by difference, into a fat-extraction flask, or into 100 ml beaker or flask, 2 to 3 g of the test sample.
- Step2. Add 7.5 to 10 ml of HCl solution so as to wash the test portion into the small bulb of the extraction flask. (as per BIS method, take 5 g of the sample and 10 ml of HCl solution).
- Step 3. Carry out a blank test simultaneously with the determination, using the same procedure and same reagents, but omitting the test portion.
- Step4. Heat by gently moving the vessel in a boiling water-bath or on a hot plate, until all the particles are entirely dissolved. Allow the vessel to stand for 20 to 60 min in the boiling water bath, shaking occasionally during the initial 15 min or keep it gently boiling over the flame or on to the hotplate for 10 min. Cool in running water.
- Step5. Add 10 ml of the ethanol and mix gently but thoroughly by allowing the contents of the flask to flow backward and forward between the two bulbs; avoid bringing the liquid too near to the neck of the flask.
- Step6. Add 25 ml of the diethyl ether, close the flask with a cork saturated with water or with a stopper wetted with water, and shake the flask vigorously but not excessively (to avoid the formation of persistent emulsions) for 1 min with the flask in a horizontal position and the small bulb extending upwards, periodically allowing the liquid in the large bulb to run into the small bulb.
- Step7. Carefully remove the cork and add 25 ml of the light petroleum. Close the flask with the rewetted cork (by dipping in water) and shake the flask gently for 30 sec.
- Step8. Centrifuge the closed flask for 1 to 5 min at a 500 to 600 rpm. If a centrifuge is not available, allow the closed flask to stand in the rack for at

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least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the flask in running water.

Step9. Carefully remove the cork and rinse it and the inside of the neck of the flask with a little of the mixed solvent so that the rinsings run into the flask or into the fat- collecting vessel. If the interface is below the bottom of the stem of the flask, raise it slightly this level by gently adding water down the side of the flask to facilitate the decantation of solvent.

Step10. Carefully decant as much as possible of the supernatant layer into the fat-collecting vessel containing a few boiling aids avoiding decantation of the aqueous layer. Rinse the outside of the neck of the Mojonnier flask with a little of the mixed solvent, collecting the rinsings on the fat collecting vessel.

Step11. Carry out a second extraction by repeating the operations described above but using only 15 ml of the diethyl ether and 15 ml of the light petroleum; use the ether to rinse the inside of the neck of the Mojonnier flask. If necessary, raise the interface to slightly above the middle of the stem of the flask to enable the final decantation of solvent to be as complete as possible.

Step12. Remove the solvents (including ethanol) as completely as possible from the flask by distillation, or from fat-collecting vessel by evaporation, rinsing the inside of the neck of the flask with a little of the mixed solvent before commencing the distillation.

Step13. Heat the fat-collecting vessel (flask placed on its side to allow solvent vapor to escape) for 1 h in the drying oven, controlled at $102 \pm 2^{\circ}\text{C}$.

Step14. Remove the fat-collecting vessel from the oven, allow to cool (not in a desiccator, but protected from the dust) to room temperature (for at least 1 h) and weigh to the nearest 0.1 mg. Do not wipe the vessel immediately before weighing.

Step15. Place the vessel on the balance using tongs (to avoid in particular, temperature variations).

Step16. Repeat the operations of heating and weighing until the mass of the fat-collecting vessel decreases by 0.5 mg or less, between 2 successive weighing. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

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Operation sheet -3.Determination of Moisture in Dried Milk

Objective: to know the moisture content of Dried Milk

Procedure

- Step1. Uncover a dish and place the dish and its lid in a hot air oven at $102 \pm 2^{\circ}\text{C}$ for 1 h. Place the lid on the dish, transfer the covered dish from the hot air oven to the desiccator. Allow it to cool to room temperature and weigh it.
- Step2. Put approximately 1 g of the dried milk sample in the dish, cover the dish with the lid and weigh the covered dish accurately and quickly.
- Step3. Uncover the dish and put it with its lid in the hot air oven maintained at $102 \pm 2^{\circ}\text{C}$ for 2 h.
- Step4. Replace the lid, transfer the covered dish to the desiccator, allow it to cool to room temperature (for approximately 30 - 45 min) and weigh it accurately and quickly.
- Step5. Heat the uncovered dish and lid in the hot air oven at $102 \pm 2^{\circ}\text{C}$ for further 1 h, replace the lid, allow the covered dish to cool to room temperature in the desiccator and weigh it. Repeat the process of drying, cooling and weighing, until the successive weighing do not differ by more than 0.5 mg. It is usually found that drying is complete after the first 2 h.
- Step6. Finally Calculate by using the equation $\text{Moisture}\% \text{by mass} = \frac{100 (M1 - M2)}{A \times M1 - M}$

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Operationsheet- 4. Determination of Salt Content in Butter by (Volhard's

Method)

Objective: to know the salt content of butter

Procedure

- step 1 Extract the salt from the residue of curd and salt by repeated washing of the Gooch crucible or filter paper with hot water, or by placing the crucible or filter paper in a beaker of hot water.
- step 2 Collect the rinsing in a 100 ml volumetric flask passing the solution through a filter paper. Allow to cool to room temperature and make up to volume.
- step 3 Take 25 ml water extract into a 250 ml conical flask, and add an excess (normally 25 to 30 ml) of 0.05 N silver nitrate solution.
- step 4 Acidify with nitric acid; add 2 ml of the indicator solution and 1 ml nitrobenzene. Mix and determine the excess of silver nitrate by titration with the potassium thiocyanate solution until the appearance of an orange tint, which persist for 15 s.
- step 5 In the same manner determine the equivalent of 25 ml or the added amount of silver nitrate as thiocyanate using the same volumes of reagents and water.
- step 6 Determine the NaCl contante by solving, $\text{NaCl, \% by mass} = \frac{23.38 \times N \times (A-B)}{M}$

M

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LAP TEST	Performance Test
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Name.....ID.....Date.....

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within 3 hours. The project is expected from each student to do it.

Task-1 Perform fat test for milk.

Task-2 Perform acidity test for milk product.

Task-3 perform moisture determination Dried Milk.

Task-4. Perform determining the salt content of butter.



LG #33

LO3. Monitor microbiological changes through the dairy products processing

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- preparing Samples for testing
- carrying out Serial dilutions
- Comparing stained specimens with reference samples
- Performing tests on cultures
- Making observations and recording data for yeasts and molds, total coli forms and staphylococci
- Carrying out Sampling and testing for inhibitory substances in milk
- Sampling whey for bacteriophage levels and interpreting the results

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- prepare Samples for testing
- carry out Serial dilutions
- Compare stained specimens with reference samples
- Perform tests on cultures
- Make observations and recording data for yeasts and molds, total coli forms and staphylococci
- Carry out Sampling and testing for inhibitory substances in milk
- Sample whey for bacteriophage levels and interpreting the results

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, see your trainer for further instructions or go back to “Operation sheets”.



Information sheet 1. Preparing Samples for testing

1.1. Sampling

Sampling of dairy products is necessary to ensure quality, safety, and specific attributes such as butterfat content. All types of dairy, cream, milk, whey, cheese – can be sampled in their various forms, whether liquid, slurry, solid or powder.

Dairy sampling and analysis is conducted to:

- Verify industry compliance to regulations
- Gather baseline information and perform assessments
- Set standards and guidelines
- Maintain consumer confidence in the safety of the food supply
- Prevent problems, health risks or fraudulent claims

Accurate analysis results require controlled, real-time data achieved through reliable, accurate and repeatable process monitoring and measuring. Producers must sample regularly to obtain representative samples, and government inspectors in many countries may take or request samples at any time.

If laboratory analysis of the samples is to be valid, it is essential to properly collect, transport and store perishable milk and milk product samples. The phrase, 'garbage in, garbage out' is relevant in relation to samples that have been compromised during collection, storage or transportation to a laboratory for analysis.

Even under the best conditions, in dairy products microorganisms can survive and grow, and chemical and physical changes can occur. Professional training of sample collectors is critical so they can be continually aware of issues that can occur during sample collection and delivery.

1.2. Sample Preparation Steps

Samples must be representative of the entire process stream, it is important that liquid dairy be adequately mixed. During sample preparation, the sample collector must ensure that microbial and chemical contamination is not introduced into the sample or process stream itself. After samples are collected, they must be protected from contamination and maintained under specified conditions, depending on the

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sample and the microbiological, chemical, and physical analysis to be performed, so they are the same when delivered to the laboratory as they were when collected.

Maintaining proper temperature is essential to preventing microbial growth and ensuring that there are no chemical or physical changes in the samples until they are analyzed. Under ideal conditions, analysis of the samples should begin within 24 hours of original collection. Collectors must ensure that all pertinent sample identification information is recorded and delivered to the laboratory as well, including the date, time and temperature of collection, and who collected the sample. Due to the legal nature of the process, when collecting samples for regulatory purposes, a chain of custody must be established to ensure only those with the proper authority handled the samples.

Because of the issues with transporting dairy within a timely period for analysis, sampling often takes place at transport trucks, receiving docks and/or transfer points using dairy samplers specifically designed for those applications.

1.3. Dairy Sampling Standards

The International Dairy Federation (IDF) currently is working on improving analytical standards for dairy sampling and analysis to:

- Detect veterinary medicinal drug residues and pesticides (insecticides, fungicides and herbicides) in milk and milk products;
- Standardize a method or methods for the determination of aflatoxin M1 and other mycotoxins in milk and milk products;
- Standardize methods of analysis for food additives in milk products, for determination of nitrate, nitrite, phosphorus and chloride in cheese and other dairy products, for elements with the exception of N, P, (PO₄)₃, polyphosphates, anions HCl and NO₃ and radionuclides;
- Study the possibilities for a suitable routine determination of vitamins A and D in milk products and develop a method for the establishment of the strength of synthetic vitamin A and synthetic vitamin D standards of different manufacturers for the routine determinations;
- Describe, evaluate and standardize microbial inhibitor and preliminary confirmation tests for the detection of antimicrobials in milk and milk products

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and the development of detection concepts, integrated detection systems, based on the methods and residues found in the various countries;

- Collect information on elements other than those cited above and analytical information on normal trace element content in milk and milk products as well as an observed higher level (from contamination) and related problems and strategies to avoid them.

Key to meeting these standards are representative samples for analysis.

1.4. Sampling of Cheese

One of the following three methods is employed to sample cheese.

A. Sampling by cutting a sector

Using a knife with a sharp blade, two random cuts are made radially proceeding from the center of the cheese/paneer towards the edge. 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.

B. Sampling by means of a trier

The cheese trier is driven obliquely into the surface of the paneer or cheese towards the center 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 9. Cheese 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.

C. Sampling by taking a whole cheese

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



Fig.5. Hole cheese sampler

1.5. Preparation of Cheese 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.

1.6. 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.

Allocation Size (N)	No. of vessels to be selected (n)
Up to 300	3
301 – 500	5
501 – 1000	7
1001 and above	10



b) For containers of more than 5 Kg and up to 20 Kg

Allocation Size (N)	No. of vessels to be selected (n)
Up to 100	2
101 – 300	3
301 – 500	4
501 and above	5

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

The allocation Size 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.



Self check 1	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What is the purpose of sampling? (3pts)
2. What are the dairy sampling standards?(3pts)
3. How can prepare samples? (3pts)

Test II: Write true if the statement is correct and false if the statement is incorrect

1. Dairy sampling and analysis is conducted to Verify industry compliance to regulations . (2pts)
2. Sampling of dairy products is necessary to ensure quality, safety, and specific attributes. (2pts)

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____

Information sheet 2. Carrying out Serial dilutions

2.1. Serial Dilution

Serial dilution generally refers to selection performed in the standard growth regimes typically used in the lab:

- flasks,
- test tubes,
- solid media,
- or 96-well plates.

Cultures are usually allowed to grow through a normal growth curve, with daily transfer of a small volume of the expanded culture into fresh medium.

Advantages of Serial Dilution

The materials necessary are typically already present in the lab and require no special engineering. Conditions can be adjusted as the experiment progresses (e.g., drug concentrations increased as drug resistance improves). Selection pressures of a number of types can be accommodated.

The easiest selections to understand are improvements to growth when maximal performance is attenuated either by exogenous or genetic means. In these cases, full growth curves may not be desired, as improved performance with respect to nutrient exhaustion or stationary phase may be separate outcomes unrelated to the main selection applied by the experimenter.

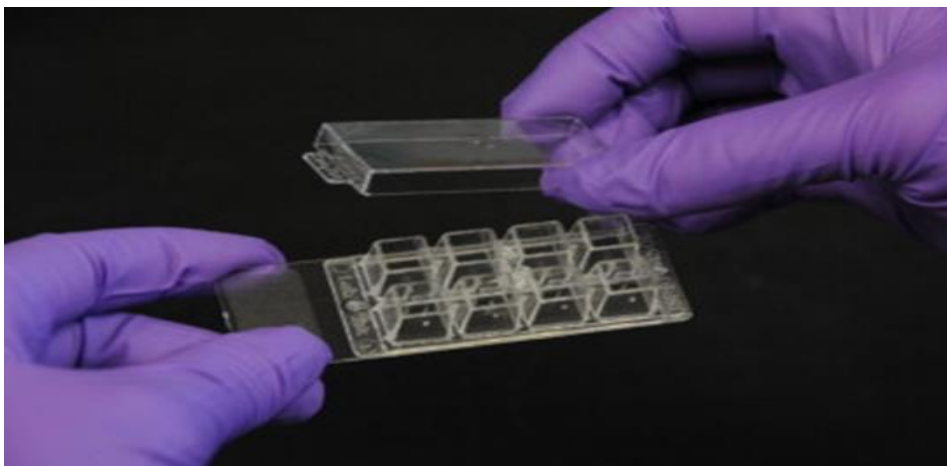


Fig.6.serial dilution plate

Here, one nutrient is lowered to the point that it uniquely runs out first and limits the saturated biomass of the culture. The relative amount of time cells spend in each

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phase of growth may change over the course of one of these experiments, particularly as lag phase shortens and maximal growth rate improves.

Prepare Serial Dilutions

Plate-based selection allows even more control over the transfer step, with visual identification of colonies. Make serial dilutions of the protein in protein dilution buffer.

- Make the dilutions 5× higher than the protein concentrations you wish to use in the binding assay (e.g., to measure binding to 10 μM protein, make a 5× stock of 50 μM).
- Aliquot 16 μl binding buffer into tubes representing each point.
- Add 4 μl of the appropriate 5× protein dilution to each reaction.
- Include a no-protein reaction (by adding 4 μl protein dilution buffer without protein) to obtain counts representing the background for the assay.
- Add at least 1000 counts of the radiolabeled nucleic acid of interest and incubate for the appropriate length of time for the reaction to reach equilibrium.

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Self check- 2.	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What is the purpose serial dilution? (3pts)
2. How can prepare serial dilution?(3pts)

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet 3. Comparing stained specimens with reference samples

3.1. Dairy Bacteriology

Microorganisms play important roles in the quality and safety of dairy products. Among these microbes bacteria are the most important one. Bacteria are single celled organisms that can only be seen with a microscope (“microorganisms”). All processes needed for life occur within a single cell. Most bacteria are considered prokaryotes. Their basic cell structure differs from cells of plants and animals (eukaryotes); for example they lack a true nucleus and have a unique cell wall. Bacteria can be found wherever life exists; some are considered useful, such as those responsible for nutrient conversion (e.g., decomposition) and food fermentations (e.g., cheese), while others are harmful, such as those responsible for food spoilage and disease.

They are classified according to their appearance and structure and by specific characteristics of their metabolism and growth, including nutrient requirements, growth temperatures, oxygen requirements, their ability to use specific substrates (e.g., certain sugars), and by specific byproducts of their metabolism. There are literally thousands of species of bacteria, but only select groups are of concern to the dairy industry. The following will describe the general characteristics important for characterizing bacteria that are common in milk and dairy products. Although not specifically covered, comments pertaining to dairy fungi (yeast & molds) are also included.

3.1.1. Appearance, Size and Shape

To see bacteria, a microscope is required, generally one with a magnification of 1000X. Bacteria are measured in microns (1 micron = 1/1000 mm = 1/25,000 inch). When a standard light microscope is used, bacterial cells are normally stained to make them easier to see. Bacteria can be observed in milk by staining a dried milk smear on a microscope slide with a specific “milk-stain” (e.g., Levowitz-Weber Stain). Bacteria grown in a petri dish (e.g., on a semi-solid nutrient “agar” media) or in a nutrient broth can be smeared and dried on a slide and stained with a simple stain (e.g., methylene blue) or complex stain (see gram-stain, next page) for observation.

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Bacteria exist in a variety of shapes, sizes and arrangements, which can be defining characteristics. Typical of what might be seen in milk and dairy products are:

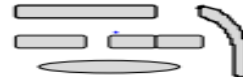
Cocci ----- Spherical cells, 0.4 - 1.5 microns. Occur as single cells, pairs, chains or clusters.

(e.g., Genera - Streptococcus, Staphylococcus).



Bacilli ----- Rod shaped, 0.5 - 30 microns. Occur as single cells, pairs or chains (e.g.,

Genera - Lactobacillus, Bacillus, Pseudomonas).

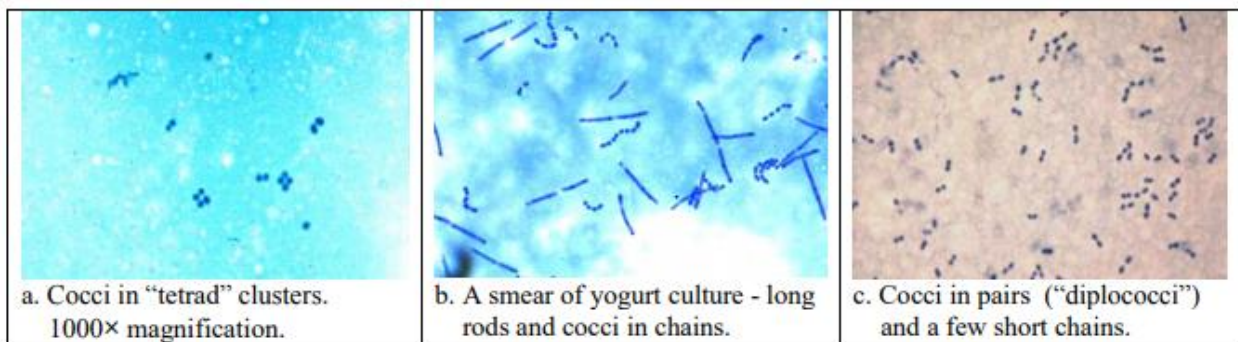


Other ----- Spiral, helical or club shaped rods of varied size. Generally are not very common in milk.

(e.g., Genus – Campylobacter (Spiral); Carnobacterium (club)).



Milk smears under the microscope stained with Levowitz-Weber Stain:



3.2. Gram-Stain Reaction

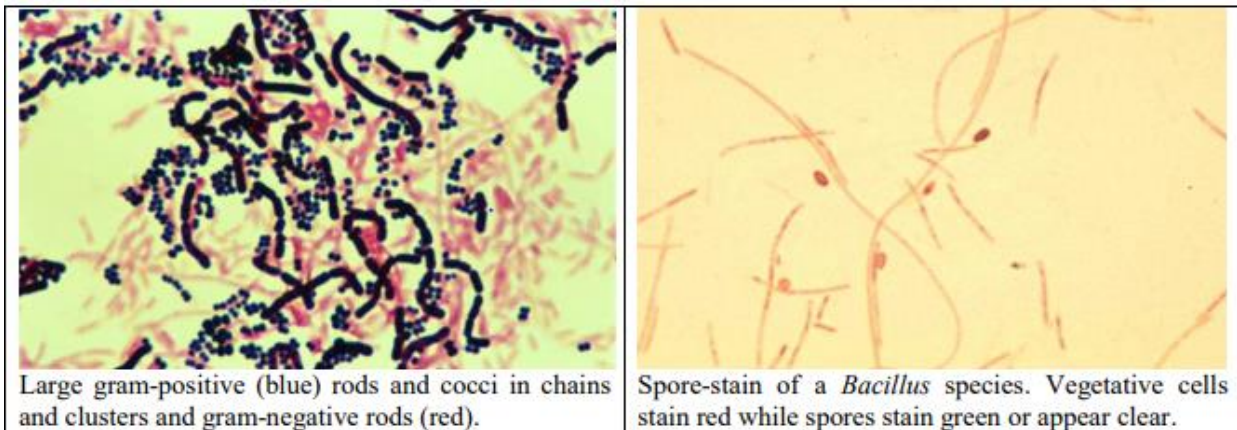
Most bacteria are classified as either “gram-positive” or “gram-negative.” This is typically determined by the gram-stain procedure, which is used to view and differentiate bacteria under the microscope; it is one of the first steps used when classifying bacteria. The gram-stain is a four step procedure with Crystal Violet (blue) and Safranin (red) as the primary stains. Depending on the characteristics of the bacteria (i.e., different cell wall structures), they will stain either blue (gram-positive) or red (gram-negative). In some cases an organism classified as “gram-positive” may stain red or appear grainy with blue and red shades. These organisms are often referred to as “gram-variable”:

Gram-positive (blue) ... e.g., Bacillus (rod); Streptococcus (cocci); Staphylococcus (cocci)

Gram-negative (red) e.g., *Pseudomonas* (rods); *E. coli* & other coliform bacteria (rods)

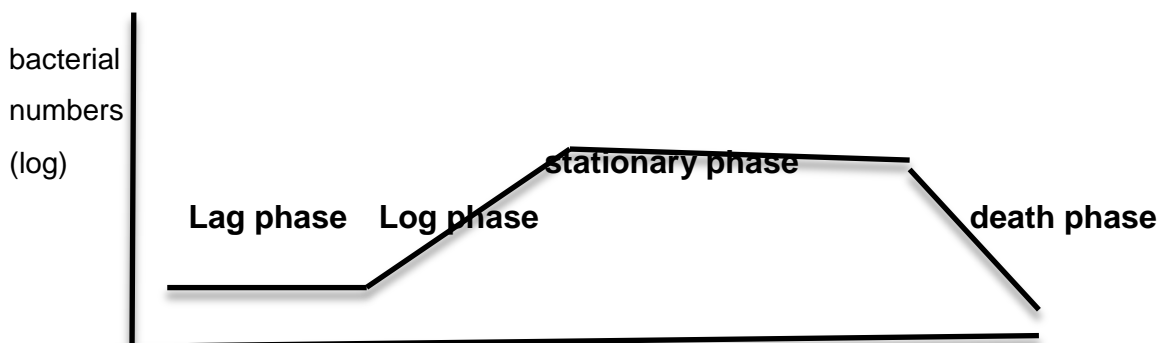
Gram-variable Stain blue or red depending on conditions; most are truly Gram-pos.

There are a few generalizations based on the gram-stain reaction that can be made of microorganisms common to dairy products. For example, **gram-negative bacteria do not survive pasteurization**; bacteria that do survive are gram-positive (but not all gram-positive bacteria survive); certain gram-negative bacteria, if present, will spoil milk faster under refrigeration compared to gram-positive spoilage organisms; specific antibiotics are more effective against gram-positive than gram-negative bacteria.



3.3. Bacterial Growth

When bacteria are introduced into a new growth environment, they often first go through a lag phase, or adjustment period, where no growth is apparent. This is followed by the active exponential or logarithmic growth phase. As the environment changes (e.g., nutrients deplete, inhibitors develop), growth will level off to a Stationary Phase, after which cells will then eventually begin to die off in the Death Phase.





3.3.1. Requirements and Conditions for Growth

Bacterial species vary widely as to what conditions are conducive for growth. Conditions that are optimum for one organism may be lethal for another. Nutrient availability, pH, moisture, the presence or absence of oxygen and other gases, the presence of inhibitors and temperature can all influence the growth of bacteria. In most cases these are not independent variables but are interactive.

3.3.2. Nutrient Requirements

Most bacteria have similar basic nutrient requirements, although different organisms will vary in their specific needs. Some bacteria require defined nutrients or conditions that make them difficult to culture. Generally, all bacteria require the following, in one form or another:

- Energy Source – from carbohydrates, proteins, lipids
- Carbon Source – from carbohydrates, proteins, lipids, carbon dioxide
- Nitrogen Source – from proteins, peptides, amino acids, ammonia, nitrates
- Vitamins – primarily water soluble B-vitamins Minerals, Metal Ions & Salts – such as potassium, phosphorus, calcium, magnesium, iron

Milk provides sufficient nutrients needed to support the growth of a large selection of microorganisms. Bacteria are capable of utilizing the proteins, fats, carbohydrates and vitamins in milk for their growth and metabolism. Different bacterial species may differ in regard to the enzymes they have or need to break down milk components.

Enzymes are biological catalysts, usually protein in nature, that facilitate a biochemical reaction, either breaking down or building a biological compound (e.g., “protease” enzymes break down proteins; “lipase” enzymes break down fats). Microbial growth and the resulting increase in microbial numbers and enzyme activity results in measurable changes in milk components and characteristics and the development of by-products that directly affect the product either in a beneficial (e.g., cheese fermentation) or harmful (e.g., milk spoilage) manner.

3.3.3. PH Requirements

The measurement of acidity and alkalinity, or pH, is expressed on a scale of 0-14, which is the negative logarithm of hydrogen ion activity of a solution or food.

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- Anaerobic – anaerobes grow only in the absence of oxygen (oxygen may be lethal) Facultative Anaerobic – facultative anaerobes can grow with or without oxygen Milk contains dissolved oxygen; thus it supports the growth of aerobic and facultative anaerobic microorganisms.

Rarely do strict anaerobes grow in milk. Some bacteria such as certain starter cultures are considered “micro aero philic,” meaning they grow best in lower levels of oxygen. Cheese may have a reduced oxygen environment due to the growth of dairy cultures and other bacteria. An oxygen-free environment may occur in the center of some cheeses, allowing the growth of certain anaerobic bacteria, some of which cause serious defects (e.g., late gas-blowing).

Clostridium botulinum is an anaerobe that produces a deadly toxin that has been associated with dairy foods on rare occasions. Influence of Inhibitors: There are a number of chemical substances that can inhibit the growth of (bacteriostatic) or kill (bactericidal) bacteria. Some examples relevant to dairy microbiology are drugs or antibiotics, lactoferrin (natural in raw milk), carbon dioxide, lysozyme (an enzyme), sanitizers, organic acids, preservatives (e.g., potassium sorbate) and natural inhibitors formed by microorganisms (e.g., nisin).

3.3.4. Temperatures for Growth

The optimum growth temperature for a bacterium is the temperature at which its generation time is shortest or it grows the fastest. Each bacterium has a minimum and maximum temperature for growth, which will vary between species and strains and with other environmental conditions. Outside of this range, growth does not occur. Bacteria are often grouped based on their optimum, minimum and maximum temperatures for growth. These are not rigid ranges, as some bacterial species may overlap into adjacent groups. General groupings of bacteria and approximate ranges are as follows:

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Table.4. Bacterial groups

Thermophilic	Min	Max	Opt
Mesophilic(heat loving)	104°F (40°C)	140°F (60°C)	122-131°F (50-55°C)
Mesophilic, Medium Temps	41°F (5°C)	122°F (50°C)	86-98o F (30-37oC)
Psychrophilic Cold Loving	32°F (0°C) or less	77°F (25°C)	<20°C

Psychrotrophic or psychrotolerant bacteria are capable of growth at 44.6o F (7°C) or less. Psychrotrophs are of primary concern to the dairy industry since they grow and cause spoilage in raw or processed dairy products commonly held under refrigeration.

a) The most commonly occurring Psychrotrophs in milk are gram-negative rods, many belonging to the genus *Pseudomonas*. Gram-negative Psychrotrophs generally do not survive pasteurization, thus they occur in processed milk and dairy products as post-pasteurization contaminants (PPC). Some strains of thermoduric bacteria are capable of growth under refrigeration storage (see below).

b) Psychrotrophs are common in the dairy environment. Milk soils (e.g., on dirty equipment) can support the growth of Psychrotrophs and other contaminants that can contaminate subsequent milk. Marginal cooling can result in relatively large numbers of these organisms in milk. Psychrotrophs might also be present in low numbers in untreated water supplies used for rinsing dairy equipment.

c) Psychrotrophic bacteria produce a variety of enzymes that cause chemical deterioration of milk resulting in off-flavors. Some of these enzymes are not inactivated by pasteurization or by other heat treatments and may continue to degrade milk products, even when the bacterium is destroyed. This has been shown to be a concern with shelf-stable (Ultra-High Temperature) milk, but there is limited information relative to conventionally pasteurized milk.

Thermoduric Bacteria: Thermoduric bacteria are a miscellaneous group of bacteria that are capable of surviving pasteurization or other heat treatments. As a general



rule, all thermophilic bacteria are gram-positive. Spore-forming bacteria (e.g., *Bacillus*, *Paenibacillus*) comprise some of the most heat-resistant bacteria.

a) Chief sources of thermophilic bacteria in milk are poorly cleaned equipment including old rubber parts, areas of milk stone build-up, separators and other difficult to clean or neglected areas (soil build-up). They may contaminate milk at the farm or at the plant. Poor pre-milking hygiene procedures (e.g., dirty cows) may also influence thermophilic levels in raw milk, especially of spore-formers.

b) Very high thermophilic counts in raw milk could result in counts in the pasteurized milk made from that milk that exceed the 20,000 cfu/ml legal limit. This is rare as counts are normally < 500 cfu/ml.

c) Most thermophilic are not Psychrotrophic, but some are. In the absence of gram-negative Psychrotrophs, certain thermophilic bacteria can grow and cause spoilage in pasteurized milk. Heat Resistant Spore-Forming Psychrotrophs (HRSP), such as strains of *Bacillus* and *Paenibacillus*, are considered common thermophilic Psychrotrophs that have become limiting factors in milk shelf-life.

Coliform Bacteria: Coliform bacteria are defined as “aerobic or facultative anaerobic, gram-negative rods, that ferment lactose with the production of acid and gas.” These characteristics allow selective detection and counting of these types of bacteria in milk and dairy products. They are considered “indicator organisms” because they are easy to detect and their presence in food & water indicate some form of contamination; e.g., the presence of “fecal” coliforms (*E. coli*) suggests the possibility of fecal contamination.

a) They are called coliforms because some members of the group (e.g., fecal coliforms) are found in the intestines (colon) of warm-blooded animals. However, many strains of coliform bacteria are common as environmental contaminants and/or are associated with other habitats (e.g., plant matter).

b) Coliforms are almost always found in raw milk, although with good production methods, the numbers can be kept very low. Sources of coliform contamination include dirty cows (manure, bedding, soil), dropped milking units, dirty equipment and, in some cases, cows with coliform mastitis.

c) Coliforms do not survive pasteurization. When detected in processed milk or dairy products, they indicate recontamination after pasteurization (Post-Pasteurization Contamination).

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3.4. Bacteria In Raw And Processed Milk

Bacteriological Standards:

- Raw Producer Milk 100,000 cfu/milliter (ml) total count (For Grade “A” Milk)
- Sour Milk 300,000 cfu/ml total count cfu = colony forming units
- Pasteurized Milk 20,000 cfu/ml total count, 10 coliform

3.4. Raw Milk

Milk, when secreted in the udder of a healthy cow is virtually sterile. As milk passes through the teat cistern and teat canal, it may be contaminated with low levels of bacteria (<1000 cfu/ml), which are generally not significant to milk quality or safety. Milk from a cow with mastitis (infection of the mammary gland) however, may harbor large numbers of the infectious bacteria. After it leaves the cow, milk can be contaminated from the exterior of the cow (dirty cows),

- The environment and/or poorly cleaned equipment.
- Poor cooling allows faster growth rates and can result in rapid increases in bacterial numbers in raw milk before it is processed.
- While the legal limit for bacteria in raw milk is 100,000 cfu/ml, the production of milk with bacteria counts less than 10,000 cfu/ml should be easily achievable for most farms.

3.5. Pasteurized Milk

Pasteurization (i.e., 161°F for 15 sec.), while designed to destroy potential pathogens in raw milk, substantially reduces the total numbers of bacteria present, increasing the shelf-life potential of milk. Unless gross recontamination has occurred, bacterial numbers in freshly pasteurized milk generally reflect the organisms that survive pasteurization (thermoduric). The legal limit for bacterial numbers in pasteurized milk is 20,000 cfu/ml. However, bacteria counts for most freshly pasteurized milks are less than 500 cfu/ml. Under proper refrigeration, the bacteria that become significant in the shelf-life and spoilage of milk are Psychrotrophic in nature. These types of organisms generally occur as postpasteurization contaminants (PPC), although a few thermoduric bacteria may be Psychrotrophs. Heat resistant Psychrotrophic spore-formers (HRSP) are often the limiting factor in milk shelf-life when PPC is prevented.

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Regardless of measures that prevent microbial contamination, the shelf-life of conventionally pasteurized milk should not be expected to exceed 21 days and still be considered “fresh.”

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Self-check 4	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Bacilli type bacteria are Spherical shaped . (3pts)
2. List two examples for each type of bacteria gram positive and gram negative. (3pts)
3. Write the difference between gram positive and gram negative bacteria. (3pts)
4. Least and discuss the bacterial growth curve. (3pts)
5. Write the Bacteriological Standards of raw and pasteurized milk.

Test II say true if the statement is correct say false if the statement is incorrect

1. Bacteria are single celled organisms that can only be seen within our naked eye. (2pts)
2. Bacteria are measured in microns. (2pts)
3. Bacilli type bacteria are Spherical shaped (2pts)

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____

Information sheet -4. Performing tests on cultures

4.1. Purpose of Test

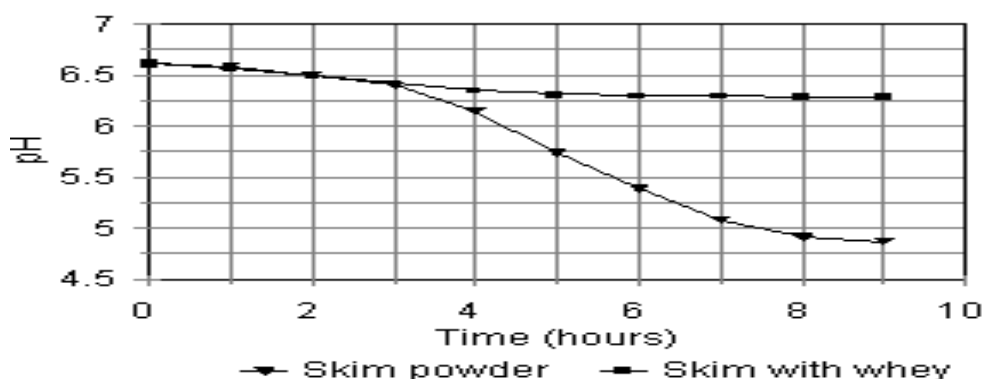
This simple test is useful to ensure that cheese cultures have adequate activity before inoculating the cheese vat. For most cheese a general rule of thumb is that the activity and amount of inoculum should be sufficient to produce a titratable acidity of about 34% lactic acid, in 10% reconstituted skim milk, after 4h of incubation at 37°C.

The test is also useful to compare types of cultures or bulk cultures prepared under different conditions. For these purposes a pH versus time chart is quite useful. A further application is to check sensitivity of the culture to bacteriophage in the plant.

Procedure

- Mix 10 g of low-heat, antibiotic-free skim milk powder in 90 ml of distilled water in a 100 ml Erlenmeyer flask.
- Sterilize at 15 lb. pressure (1.05 k Pa.) for 10 min.
- Cool to 37C.
- Inoculate with 3.0 ml starter or other amount as appropriate. Rinse pipette twice by drawing the sterile milk into it.
- Incubate at 37C for at least 4 h. Longer if desired for pH versus time profile.
- Check pH at 30 min. intervals.
- Titrate 17.6 ml with N/10 sodium hydroxide (NaOH) using 1 ml phenolphthalein. Divide the required ml of NaOH by 2 the btain titratable acidity in units of percent lactic acid.
- Record starter activity as follows: Active, over 0.34% Slow 0.26 to 0.30%.

Culture Activity: pH vs Time





4.2. Detection of Bacteriophage

The following tests are based on the principle that bacteriophage specific to the culture in use will be present in high numbers in the cheese whey. Therefore, by monitoring whey for the presence of phage "a dead vat" on subsequent days can be avoided.

4.3. Culture Activity Test

The culture activity test described above can be used to detect the presence of phage in cheese whey.

- Prepare 300 ml of reconstituted skim milk and place 99 ml in each of three beakers.
- Add 1 ml of whey to Beaker 1 (100 x dilution), then transfer 1 ml from Beaker 1 to Beaker 2 (10,000 x dilution) and finally, transfer 1 ml from Beaker 3 to Beaker 4 to make a 1 million times dilution.
- Add culture and monitor pH.

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**Self check 4****Written test**

Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. Write the procedures to test culture.(6)
2. Test II say true if the statement is correct say false if the statement is incorrect
3. Culture test is useful to ensure that cheese cultures have adequate activity before inoculating the cheese vat.(2pts)
4. culture activity test used to detect the presence of phage in cheese whey.(2pts)

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet-5. Making observations and recording data for yeasts and molds, total coli forms and staphylococci

5.1. Microbiological Test Procedures

Milk, being a good medium for growth and proliferation of a variety of microorganisms such as bacteria, moulds & yeast and their toxins, needs to be stringently screened before use in order to prevent transmission of these microbes to consumers through milk. The most commonly used tests recommended by FSSA and the test recommended by this assessment for state and district laboratories are given below:

5.1.1. Total plate count or Standard plate count

Total plate count results reflect the number of colonies that can emerge under the given physical and chemical conditions (atmosphere, temperature, pH, available nutrients, and presence of growth inhibitory compounds). Colonies are aggregates of living microbial cells, and hence, the results cannot be compared with those from direct counts. Plate counts under estimate the presence of microorganisms, as quiescent, viable but not culturable, and non culturable microorganisms are omitted from the count.

Materials Required

Diluent: 0.1% Peptone or Phosphate buffer (90 or 99 ml, 9 ml)

- Media: Plate count agar (PCA) medium (pH 7.0 at 25^o C); autoclaved at 121^o C for 15 minutes
- Pestle & mortar, Petri dishes, pipettes, incubator (35^o C) & Marker

Procedure

Sampling: Collect the sample randomly from entire lot. According to FSSA sampling 5 packets (500 ml) of samples will be collected from entire lot. Sample will be stored at refrigeration temperature (2-7^oC) until analysis.

Preparation of the test sample: 100-150 ml of the sample will be poured into the sterile sample bottle from each packet. Opened packet will be sealed and kept at refrigeration temperature (2-7^oC).

Serial dilution and plating

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- Mark the tubes and petri dishes for batch no., sample no., parameter, dilution etc.
- Pipette out 1 mL of diluted sample from 1:10 dilution bottle into 9 mL of diluent tube
- Pipette out 1 mL from each dilution into respectively marked dilution plates
- Pour the PCA (50oC) medium into the plates and allow it to solidify
- Incubate the plates at $35 \pm 2^{\circ}\text{C}$ for 48–72 hrs

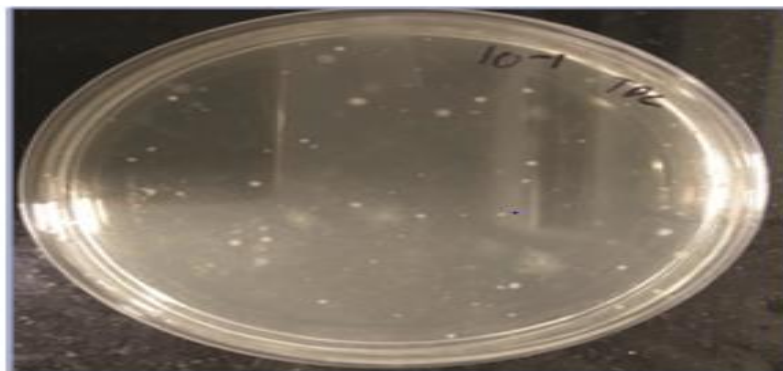


Fig: Typical colonies on plate count agar

Counting & Calculation: After incubation, retain dishes containing not more than 300 colonies at two consecutive dilutions. It is necessary that one of these dishes contains at least 15 colonies. Calculate the number N of micro-organisms per milliliter or per gram of product, depending on the case, using the following equation:

$$N = \frac{\sum C}{(n_1 + 0.1n_2)d}$$

Where $\sum C$ is the sum of colonies counted on all the dishes retained; n_1 is the number of dishes retained in the first dilution; n_2 is the number of dishes retained in the second dilution; d is the dilution

Factor corresponding to the first dilution. Round the result calculated to two significant figures. Take as the result the number of micro-organisms per mill liter or per gram of product, expressed as a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10.

5.2. Yeast and mould counts

Total Yeast and Mold Counts (TYMC) are used to detect and quantify the amount of fungal growth and allow for identification of viable yeast and mold species present. The amount of fungi is reported as the number of colony forming units (CFUs).



Materials Required

Diluent: 0.1% peptone or Phosphate buffer (90 or 99 ml, 9 ml)

Media: Yeast Extract-Dextrose Chloramphenicol (YEDC) Agar (pH 5.4); Autoclaved at 121°C for 15 minutes , 10% tartaric acid solution/ 1% lactic acid 0.25 % of sterile sodium propionate solution, Pestle & mortar, Petri dishes, Glass pipettes, Incubator (25°C) and Marker.

Procedure

Sampling: Collect 100 – 150 gm. of milk and milk product from the entire lot randomly and store the sample at refrigeration temperature (27°C) until analysis.

Serial dilution & plating

- Weigh 10 of milk and milk product sample aseptically and add into pestle containing 90 ml potassium phosphate buffer (pH 7.0) or 0.1% peptone water
- Triturate the sample in pestle by using mortar along with diluent for few minutes and pour it back into the dilution bottle (1:10 dilution/ 1st Dilution) for further dilution
- Pipette out 1 ml of diluted sample from 1st Dilution into 9 ml of diluent and it is treated as (1:100 or 2nd dilution).
- At the same time pipette out 1 ml diluted sample from respective dilution 1:10/ 1: 100) dilution into respectively marked petri dishes
- Pour about 15 ml of the YEDCA medium, previously melted and maintained at 45±1oC in a water-bath, from a culture bottle into each petri dish.
- Make a separate count of the yeast colonies, which usually will be characterized as smooth, moist, elevated or surface colonies.
- Carefully mix the inoculum with the medium and allow the mixture to solidify by leaving the petri dishes to stand on a cool - horizontal surface. Prepare a control plate, with 15 ml of the medium, to check its sterility
- The time elapsing between the end of the preparation of the initial suspension (or of the 10-1 dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min. At the time of addition the pH of the medium should be reduced to 3.5 using 10% tartaric acid or 1% lactic acid

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- After counting the typical yeast colonies, count the mould colonies. Mould colonies are easily recognized by their profuse growth of hyphae. If only yeast counts are required, add 0.25 % of sterile sodium propionate solution to the plate at the time of pouring to inhibit the growth of moulds

If necessary, carry out a microscopic examination in order to distinguish, according to their morphology, the colonies of yeasts and moulds from colonies of bacteria. Generally, it is desirable to differentiate between moulds and yeasts. It is advisable to examine the plates at the end of three days for yeast colonies as they are likely to be overgrown by mould growth.

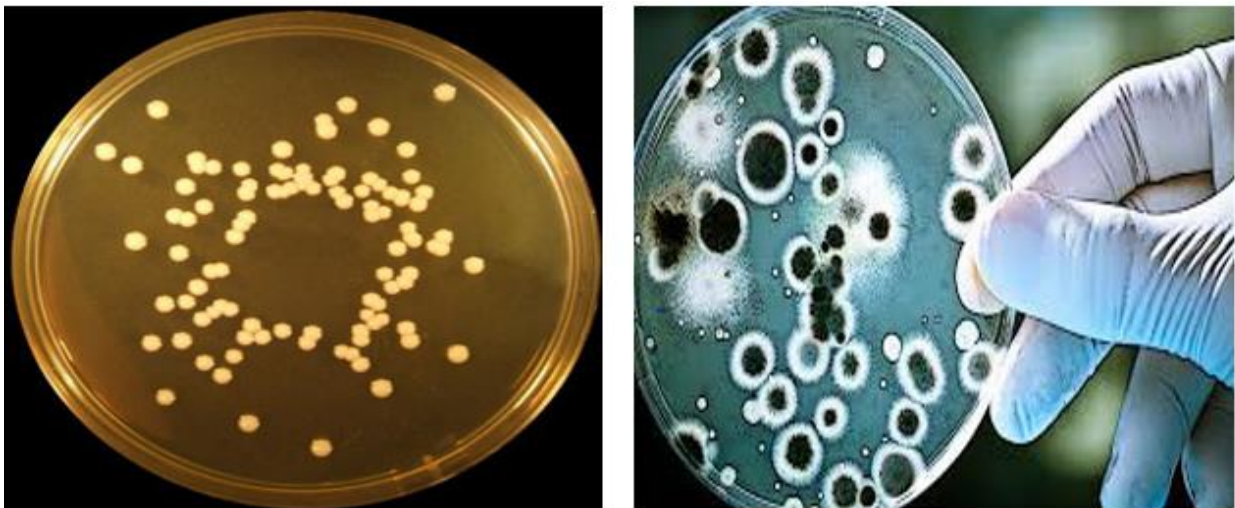


Fig: Plates showing yeast and mould colonies

Interpretation

Count the colonies on each plate after 3, 4 and 5 days of incubation. After 5 days, retain those plates containing fewer than 150 colonies. If parts of the plates are overgrown with moulds, or if it is difficult to count well-isolated colonies, retain the counts obtained after 4 or even 3 days of incubation. In this event, record the incubation period of 3 or 4 days in the test report.

Expression of results

Use counts from plates containing fewer than 150 colonies. The number of yeasts and moulds per gram or per milli litre is calculated according to the equation described in above. If there are no colonies on plates from the initial suspension, if the initial product is solid, the number of yeasts and moulds per gram of product should be reported as fewer than 10. If there are no colonies on plates from the test

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sample. If the initial product is liquid, the number of yeasts and moulds per millilitre of product should be reported as less than 1.

5.3. Coliform count

Coliforms are a group of Gram-negative rod-shaped bacteria that have similar biochemical characteristics and are not a single species of microorganism. They are used to monitor the quality of milk being able to ferment lactose with the production of acid and gas within 48 hr at 35°C and grow with or without oxygen. These are usually present in small number in raw milk. It is a simple test and easy to conduct. Absence of coliforms in 1:100 dilutions in raw milk and in 1:10 dilution of pasteurized milk is accepted as a satisfactory quality. The presence of E. coli is a proof that contamination from excreta has occurred.

Materials required are A-1 Medium, EC medium, m-FC medium, Membrane filtration unit; Test tubes/ Sampling bottle; Incubator (37°C and 45°C)

Procedure-1: Single-step procedure

- Dilutions of the sample are inoculated into fermentation tubes of A-1 medium
- Tubes showing growth plus gas are considered positive for fecal coliforms
- The tubes are first incubated for 3 hrs at 35°C and then transferred to a 44.5°C water bath for an additional 21 hrs of incubation.

Procedure-2: Multiple Fermentation Tube (MFT) Technique

- Cultures from positive tubes from the Presumptive (total) coliform test are inoculated into fermentation tubes of E. coli medium
- Incubated at 44.5°C for 24 hrs.
- Tubes showing growth with gas production are considered confirmed positives.

Procedure-3: Membrane Filtration (MF) technique

Samples are filtered onto membranes as in the total coliform test; the membranes are placed onto plates of m-FC medium, sealed in water-tight plastic bags, and submerged in a 44.5°C water bath incubator for 24 hrs. Colonies with a characteristic fecal coliform appearance are then counted and fecal coliform density is computed.

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Fig: Typical colonies ion VRBL Agar

5.4. Escherichia coli

Conventional method for the enumeration of E. coli.

Media and reagents ; Lauryl tryptose (LST) broth, levine's eosin-methylene blue (L-EMB) agar, MR-VPbroth, Butterfield's phosphate-buffered water, Kovacs' reagent, Voges-Proskauer (VP) reagents, Methyl red indicator ,Violet red bile agar (VRBA), Peptone Diluents, 0.1% & Brilliant Green Lactose Bile Broth Materials, Test sample, 1% peptone, MacConkey broth medium, MacConkey agar medium, Eosin methylene blue lactose agar, Tergitol-7 agar, Kovac's reagent, Methyl red, alpha-naphthol, Potassium hydroxid, Incubator and Pipette

Procedure

- Blend the sample in a sterile blender jar for 2 minutes or macerate with sterile sand in a sterile mortar using approximately 200 mL of diluting fluid per 25 g of the sample
- The diluting fluid for preparing the homogenate should be at 1% peptone (ISO 6887 solution in water
- Inoculate 1 mL of the blended or macerated sample into 10 mL of single strength MacConkey broth medium. If the numbers of organisms are assumed to be very small, inoculate 10 mL of double strength MacConkey broth medium.
- Also streak loop full on to MacConkey agar medium, eosin methylene blue lactose agar, and if available Tergitol-7 agar
- Incubate all the inoculated media at 37°C overnight
- If there is growth with fermentation of lactose in the MacConkey broth medium streak out a loop full on to each of the solid media, and incubate at 37°C overnight.

Test for Identification

Pick out and mark as many suspected colonies from the solid media as possible, but not less than 5, to investigate. The suspect colonies are smooth and are lactose fermenting on Mac Conkey agar and on eosin ethylene blue lactose agar, and are yellow colonies surrounded by yellow zones on Tergitol-7 agar medium.

Procedure for enumeration:

Preparation of Sample

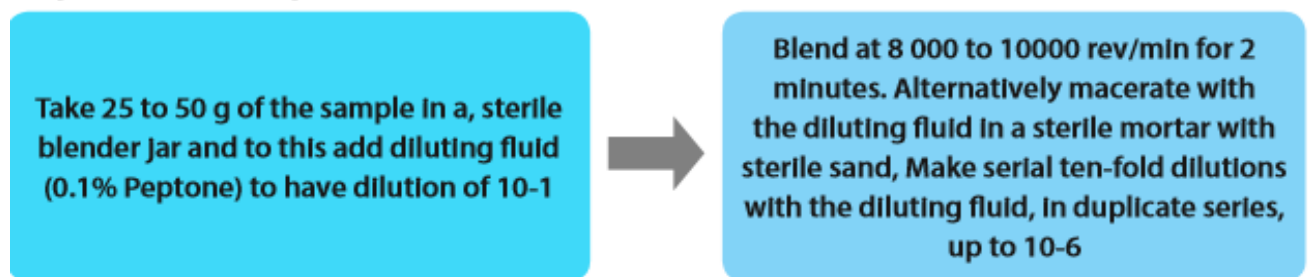
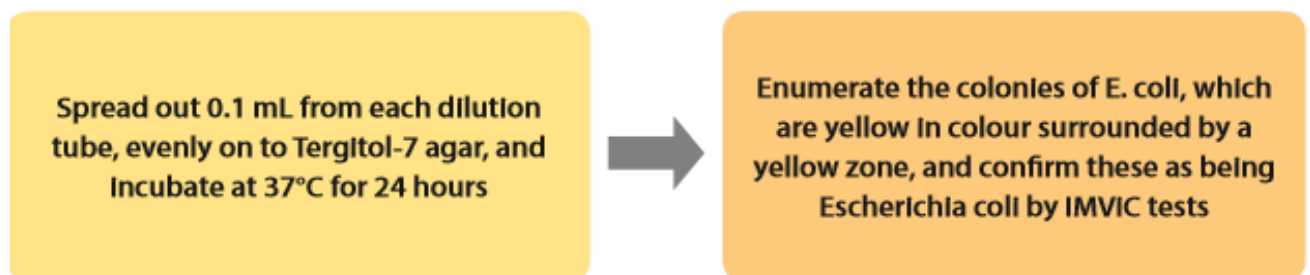


Plate Count:



The number of viable colonies of *E. coli* per gram of sample shall be determined by multiplying by the dilution factor(s) and dividing by the mass of the sample. If Tergitol-7 agar is not in use, then MacConkey agar plates or eosin methylene blue lactose agar plates may be used.

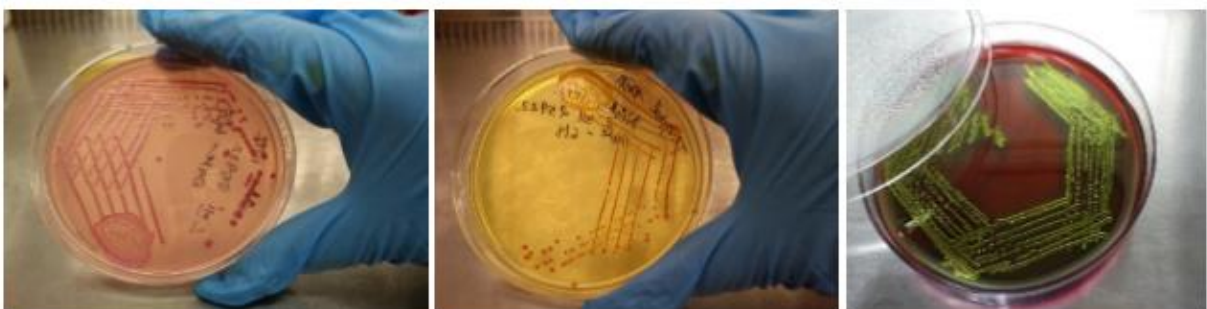


Fig: E. coli on MacConkey Agar, Tergitol 7 Agar and EMB Agar



5.5. Staphylococcus aureus

Highly pathogenic strains of Staphylococcus aureus can cause disease in both humans and animals. In animal species, including ruminants, S. aureus may cause severe or sub-clinical mastitis. Dairy animals with mastitis frequently shed S. aureus into the milk supply which can lead to food poisoning in humans.

Materials required

Incubator, Drying cabinet or incubator, Water bath, Test tubes, Flasks or bottles with screw caps, Petri dishes, straight wire and Pasteur pipette, Total-delivery graduated pipettes, Spreaders, pH Meter.

Initial suspension and dilutions;

- Agitate the sample thoroughly by inverting the sample container 25 times
- Pipette out 1mL or 10 mL of the sample with a sterile pipette and add to 9mL or 90 mL of diluents
- Shake this primary dilution with a movement of about 300 mm for 7s manually or using a mechanical agitator to obtain a 10⁻¹ dilution

Inoculation

- Transfer 0.1 ml of the test sample if liquid, or 0.1 ml of the initial suspension (10⁻¹ dilution) in the case of other products, to each of two Baird Parker agar (BPA) plates.
- Repeat the procedure for the 10⁻² dilution and for further decimal dilutions, if necessary.
- If, for certain products, it is desirable to count low numbers of coagulase-positive staphylococci, the limits of detection can be raised by a factor of 10 by inoculating 1.0 ml of the test sample if liquid, or 1.0 ml of the initial suspension for other products, either on the surface of one large agar plate (140 mm) or on the surface of three small agar plates (90 mm). In both cases, prepare duplicates by using two large plates or six small ones.
- Carefully spread the inoculum as quickly as possible over the surface of the agar plate, trying not to touch the sides of the dish, using the spreader. Allow the plates to dry with their lids on for about 15 min at laboratory temperature.

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Incubation

Invert the plates prepared and incubate them for 24 h \pm 2 h then reincubate for a further 24 h \pm 2 h in the incubator at 35°C or 37°C).

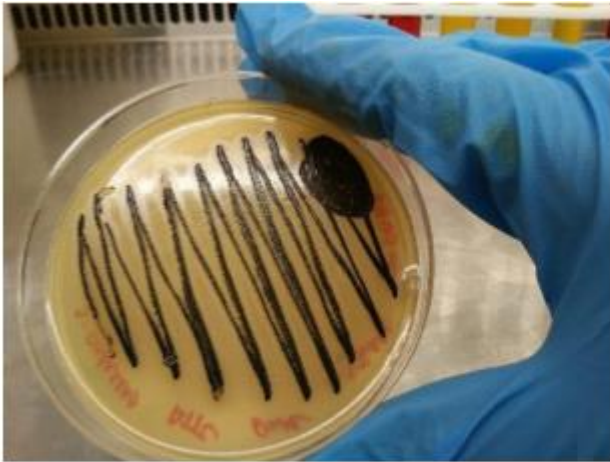


Fig: Staph. aureus on Baird Parker Agar

Selection of plates and interpretation

- After incubation for 24 h \pm 2 h, mark on the bottom of the plates the positions of any typical colonies present
- Re-incubate all plates at 35°C or 37°C for a further 24 h \pm 2 h, and mark any new typical colonies. Also mark any atypical colonies present. Take for enumeration only those plates that contain at the maximum 300 colonies with 150 typical and/or atypical colonies at two successive dilutions. One of the plates shall contain at least 15 colonies.
- Select for confirmation a given number A (in general 5 typical colonies if there are only typical colonies, or 5 atypical colonies if there are only atypical colonies, or 5 typical colonies and 5 atypical colonies if both types are present, from each plate).



Self-check 5.	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. write the materials required to perform total plate count or standard plate count. (3pts)
2. how can calculate the number of micro-organisms per milliliter or per gram of product. (3pts)
3. Enumerate the number of viable colonies of E. coli.(3pts)

Test II say true if the statement is correct say false if the statement is incorrect

1. Total plate count results reflect the number of colonies that can emerge under the given physical and chemical conditions.(2pts).
2. if the initial product is solid, the number of yeasts and moulds per gram of product should be reported as fewer than 1. (2pts)
3. If the initial product is liquid, the number of yeasts and moulds per millilitre of product should be reported as less than 10. (2pts)
4. Cheese culture (0.2 ml) is added to whey dilution tubes and to a control tube for each strain.(2pts)

Test III fill the blank with appropriate words

_____1. are aggregates of living microbial cells, and hence, the results cannot be compared with those from direct counts.

_____2.used to detect and quantify the amount of fungal growth and allow for identification of viable yeast and mold species present.

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet-6. Carrying out Sampling and testing for inhibitory substances in milk

6.1. Inhibitory substances in milk

Milk collected from producers may contain drugs and/or pesticides residues. These when present in significant amounts in milk may inhibit the growth of lactic acid bacteria used in the manufacture of fermented milk such as Mala, cheese and Yoghurt, besides being a health hazard. The suspected milk sample is subjected to a fermentation test with starter culture and the acidity checked after three (3) hours. The values of the titratable acidity obtained are compared with titratable acidity of a similarly treated sample which is free from any inhibitory substances.

Materials

- test tubes
- Starter culture
- 1ml pipette
- water bath
- material for determination of titratable acidity

Procedure

- Three test tubes are filled with 10 ml of sample to be tested and three test tubes filled with normal milk.
- All tubes are heated to 90 °C by putting them in boiling water for 3 - 5 minutes.
- After cooling to optimum temperature of the starter culture (30,37, or 42°C), 1 ml of starter culture is added to each test tube, mixed and incubated for 3 hours.
- After each hour, one test tube is from the test sample and the control sample is determined.

6.2. Assessment of results

If acid production in suspected sample is the same as the normal sample, then the suspect sample does not contain any inhibitory substances. If acid production as suspect sample is less than in the normal milk sample, then, the suspect sample contains antibiotics or other inhibitory substances.

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Self-check -6	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

1. What are inhibitory substances in dairy product.(4)
2. How can determine the presence of inhibitory substance?(2pts)
3. List the materials used to perform inhibitory test.(3pts)

Test II say true if the statement is correct say false if the statement is incorrect

1. drugs and pesticides residues in Milk disturb the growth microbes. (2pts)
2. If acid production as suspect sample is less than in the normal milk sample, then, the suspect sample contains antibiotics or other inhibitory substances.(2pts)

Note: Satisfactory rating - 13 points Unsatisfactory - below 13 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet-7. Sampling whey for bacteriophage levels and interpreting the results

7.1. Detection of Bacteriophage

Bacteriophage are virus that infects bacteria. The following tests are based on the principle that bacteriophage specific to the culture in use will be present in high numbers in the cheese whey. Therefore, by monitoring whey for the presence of phage "a dead vat" on subsequent days can be avoided.

7.2. Culture Activity Test

The culture activity test used to detect the presence of phage in cheese whey. Prepare 300 ml of reconstituted skim milk and place 99 ml in each of three beakers. Add 1 ml of whey to Beaker 1 (100 x dilution), then transfer 1 ml from Beaker 1 to Beaker 2 (10,000 x dilution) and finally, transfer 1 ml from Beaker 3 to Beaker 4 to make a 1 million times dilution. Add culture and monitor pH as described in section

4.1.. Bromocresol Purple (BCP) Phage Inhibition Test

This test is quite simple to perform, and produces more accurate results than the culture activity test.

Prepare Materials

- BCP stock solution (1 g/100 ml water)
- Test tubes containing 9.9 ml sterile BCP-milk (5 ml BCP stock solution/litre milk)
- 30-32C water bath or heating block
- 1 ml graduated pipettes
- Membrane filter (0.45 u) -- optional
- Disposable syringe -- optional
- Clinical centrifuge -- optional
- Whey sample for phage testing
- Freshly grown culture, frozen syringe, or frozen can of each strain

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Add Whey to BCP Milk and Make Dilutions

Transfer 0.1 ml of fresh (or filter-sterilized) whey to the first dilution tube (10-2) and mix well. Transfer 0.1 ml from the first to the second dilution tube and mix well. Repeat process for the third dilution tube. (If unfiltered whey is used, a control tube containing BCP milk and whey only, must be prepared. This control tube tests for the presence of active culture in the whey that could mask phage inhibition of a strain.) Whey samples should be refrigerated immediately after collection and held cold until tested for phage.

Add Culture to Control and Whey Dilution Tubes

Cheese culture (0.2 ml) is added to whey dilution tubes and to a control tube for each strain. If you are using direct-to-the-vat culture, dilute 1 ml of culture in 9 ml of milk and then add 0.2 ml of the mixture to the dilution tubes. The control tube contains only BCP milk and culture--NO whey. The control tube serves to show starter strain inhibition by colour comparison with the other tubes.

Incubate Tubes and Interpret Results. Incubate both control and dilution tubes for 6 hours at 30-32⁰C. Compare the colour of the whey dilution tubes to that of the control tube. Ignore coagulation. An inhibited culture will produce sufficient acid to turn the BCP dye from blue to yellow.

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Self -check 7	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Questions

- 1. How can determine bacteriophage.(4)
- 2. What is bacteriophage?(2pts)

Test II say true if the statement is correct say false if the statement is incorrect

- 1. Culture activity test is quite simple to perform, and produces more accurate results than the Bromocresol Purple. (2pts)
- 2. Cheese culture (0.2 ml) is added to whey dilution tubes and to a control tube for each strain..(2pts)

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Operation sheet -1 Procedure of performing Serial dilution & plating

Objective: to determine the colony forms

Procedure:

1. Weigh 10 of milk and milk product sample aseptically and add into pestle containing 90 ml potassium phosphate buffer (pH 7.0) or 0.1% peptone water
2. Triturate the sample in pestle by using mortar along with diluent for few minutes and pour it back into the dilution bottle (1:10 dilution/ 1st Dilution) for further dilution
3. Pipette out 1 ml of diluted sample from 1st Dilution into 9 ml of diluent and it is treated as (1:100 or 2nd dilution).
4. At the same time pipette out 1 ml diluted sample from respective dilution 1:10/ 1: 100) dilution into respectively marked petri dishes
5. Pour about 15 ml of the YEDCA medium, previously melted and maintained at $45\pm 1^{\circ}\text{C}$ in a water-bath, from a culture bottle into each petri dish.
6. Make a separate count of the yeast colonies, which usually will be characterized as smooth, moist, elevated or surface colonies.
7. Carefully mix the inoculum with the medium and allow the mixture to solidify by leaving the petri dishes to stand on a cool - horizontal surface. Prepare a control plate, with 15 ml of the medium, to check its sterility
8. The time elapsing between the end of the preparation of the initial suspension (or of the 10-1 dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min. At the time of addition the pH of the medium should be reduced to 3.5 using 10% tartaric acid or 1% lactic acid
9. After counting the typical yeast colonies, count the mould colonies. Mould colonies are easily recognized by their profuse growth of hyphae. If only yeast counts are required, add 0.25 % of sterile sodium propionate solution to the plate at the time of pouring to inhibit the growth of moulds

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Operation sheet 2 Procedure perform culture test

Objective: to know the culture performance

Procedure:

1. Mix 10 g of low-heat, antibiotic-free skim milk powder in 90 ml of distilled water in a 100 ml Erlenmeyer flask.
2. Sterilize at 15 lb pressure (1.05 kPa.) for 10 min.
3. Cool to 37C.
4. Inoculate with 3.0 ml starter or other amount as appropriate. Rinse pipette twice by drawing the sterile milk into it.
5. Incubate at 37C for at least 4 h. Longer if desired for pH versus time profile.
6. Check pH at 30 min. intervals.
7. Titrate 17.6 ml with N/10 sodium hydroxide (NaOH) using 1 ml phenolphthalein. Divide the required ml of NaOH by 2 the obtain titratable acidity in units of percent lactic acid.
8. Record starter activity as follows: Active, over 0.34% Slow 0.26 to 0.30%.

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LAP TEST	Performance Test
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Name.....ID.....Date.....
....

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within **2** hours. The project is expected from each student to do it.

Task-1 Serial dilution & plating

Task-2 perform culture test



LG #12	LO4. Carry out testing and interpret results to make adjustments to dairy products processing processes
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Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Using raw milk quality tests, tactile and visual senses during cheese making.
- Evaluating organoleptic properties of final dairy products processing
- Recording and interpreting test results for dairy products
- Documenting recommended specifications for physical, chemical and microbial properties
- Evaluating yield efficiency for process control parameters
- Referring specifications against test data
- Implementing changes to cheese making process

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Use raw milk quality tests, tactile and visual senses during cheese making.
- Evaluate organoleptic properties of final dairy products processing
- Record and interpreting test results for dairy products
- Document recommended specifications for physical, chemical and microbial properties
- Evaluate yield efficiency for process control parameters
- Reference specifications against test data
- Implement changes to cheese making process

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the “Information Sheets”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-checks” which are placed following all information sheets.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-checks).
6. If you earned a satisfactory evaluation proceed to “Operation sheets
7. Perform “the Learning activity performance test” which is placed following “Operation sheets” ,
8. If your performance is satisfactory proceed to the next learning guide,
9. If your performance is unsatisfactory, ask your trainer for further instructions or go back to “Operation sheets”.



Information sheet-1 Using raw milk quality tests, tactile and visual senses during cheese making

1.1. Introduction

Testing milk for organoleptic characteristics is also called sensory testing and uses the normal senses of sight, smell and taste in order to determine the overall quality. The result of this test is obtained immediately and is of minimum cost. This type of testing can be very reliable if carried out by an experienced person. Testing for organoleptic characteristics is used as a screening test to determine whether to accept the milk or test the milk further.

1.2. Appearance

The colour of cow milk should be slightly yellowish white; a different colour may indicate milk, which is unsuitable for processing. In order to judge the appearance of the milk, remove the lid of the milk container and note the appearance of the surface of the milk and the lid, note any abnormal colour of the milk, visible dirt and particles, changes in viscosity etc. After emptying the container, inspect the inside of the lid and the container for visible dirt and impurities. Take note of the following appearances:

- Visible dirt and impurities can indicate that the milk is produced under unhygienic conditions.
- Yellow milk can indicate pus or colostrum.
- Reddish milk could indicate that there is blood in the milk.
- A “blue thin” colour and a thin and watery appearance can indicate that the milk contains added water or skimming (fat removal).
- Large clots can indicate sour milk or mastitis milk.
- Small white clots or grains can indicate either Mastitis milk or milk adulterated with flour and / or skim milk powder.

1.3. Taste and smell

A bad smell or taste of the milk may be caused by bacteria, chemical reactions or by other flavors absorbed by the milk. Judging the quality of milk by its taste and smell requires considerable skill, which can only be acquired by practice.

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The taste of milk is more permanent and easy to define than smell. Taste raw milk only after making sure that it is from healthy animals. Any abnormal smell is noticed by inhalation of air standing above the milk in the upper part of the milk can. Samples for tasting must be spread around in the mouth in order to identify the taste. In addition to these basic tastes, the mouth also allows us to distinguish characteristics such as coolness, warmth, sweetness, etc. The different abnormal flavors are described as follows:

- Acid flavors are easily detected by smell and taste. The flavor is caused by the growth of acid-producing bacteria that reduce lactose to lactic acid.
- Rancid and bitter flavors: a pure bitter flavor can be detected by taste only. The rancid flavor can be detected by both the senses of smell and taste and is caused by lipolysis (deterioration) of fat.
- Feed flavors like garlic, onion, beets, poorly made silage, certain plants and pastures can cause off flavors to milk.
- Flat flavors are quite easy to detect. A very slight oxidized flavor suggests flat flavor as well as low solids and/or low fat milk.
- Malty Flavors are very suggestive of malt. The flavour is caused by the growth of the bacteria *Streptococcus lactis* var. *maltigenes*.
- Oxidized flavours are sometimes described in such terms as “oily”, “stale”, “tallow”, “cardboard” or “sunshine”. The oxidized flavour is characterized by a quick taste reaction.
- Salty flavours are easy to detect; and often associated with milk from cows in an advanced stage of lactation or mastitis milk. It is caused by an increase in chlorine and decrease in lactose content.
- Unclean flavours suggest mustiness, staleness and foul stable air.
- Other flavours such as drugs, disinfectants and detergents can also be causes bad smell and flavour.

1.4. Physical and chemical characteristics

1.4.1. Lactometer test for water addition

This method is used to determine added water, level of solids or removal of fat. With a lactometer the specific density of milk is measured. The specific gravity of the milk varies according to the proportions of fat, SNF and water. At 15 °C, the normal

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density of the milk ranges from 1.028 to 1.034 g/ml, whereas water has a density of 1.0 g/ml.

Readings between 1.028 and 1.033 are considered normal and are sometimes recorded as degrees using the last two figures, i.e. 28 and 33. It is best to combine the lactometer reading with a fat test if the results of the fat test are low and the density is high (e.g. 1.035), then the milk might have been skimmed. If the results of the fat test are low and the density is low (e.g. 1.027), then water might have been added to the milk. You can use the lactometer reading together with the fat content to estimate the SNF content of the milk. Make sure you adjust readings according to the temperature as indicated in table 3. Please take note that at high altitude milk boils at a lower temperature.

Table 3: Temperature adjustments for lactometer readings

Temp°C	17	18	19	20	21	22	23	24
Correction	-0.7	-0.5	-0.3	0	+0.3	+0.5	+0.8	+1.1

For example if the lactometer reading is 30.5 and the temperature is 23°C:

$$\text{Corrected lactometer} = L_c = 30.5 + 0.8 = 31.3$$

1.5. Freezing point test for water

This method used to determine water content, confirmation of density test. Milk and water have different freezing points; therefore added water in milk can be detected by measuring the freezing point of the sample. Water has a freezing point of 0 °C, whereas 'normal' milk has a freezing point of around -0.540 °C, due to dissolved components (mainly lactose and salts). If milk has failed the lactometer test, a freezing point test can confirm the findings. The milk sample is measured using the Cryoscope together with a sample of pure water as per the suppliers operating instructions.

The difference between the two samples is called

- The Freezing Point
- Depression (FPD).

At a measured freezing point of less than -0.530 °C, there is an indication that water has been added to the milk. There may be considerable variation in freezing points

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due to variations between animals. In practice, the freezing-point depression of quantities of 900 liters or more is unlikely to be less than $-0.540\text{ }^{\circ}\text{C}$ while milk from a single animal is unlikely to have a freezing-point depression of less than $-0.530\text{ }^{\circ}\text{C}$.

The addition of sugar, salt or milk powder to mask addition of water will not be detected by the freezing point test. The development of acidity in a sample of milk causes an increase in the freezing-point depression, which might mask, partially or completely, the contrary effect of added water. A statement of the titratable acidity of the sample, should therefore accompany the freezing-point test at the time of testing

1.6. Sediment test

This method used to judge cleanliness of milk. The amount of dirt in milk may be an indicator of the hygienic conditions during milk production and handling. By filtering milk through a white disc, these dirt particles become visible. The presence of sediment however, does not necessarily indicate the bacteriological quality of the milk.

For the purpose of comparison, it is convenient to use about five prepared standard discs to classify the milk. Milk can be then classified according to the five discs grades: Excellent, Good, Fair, Poor and very bad. Any hair, files, pieces of hay or straw, or any large particles of dirt shall be reported separately. Be aware that the lack of sediment is not always indicative of ideal conditions, because the milk might have been strained at the farm.

1.7. Temperature test

Test for screening or grading system. Most bacteria prefer to grow in the temperature region of $20\text{ }^{\circ}\text{C}$ to $45\text{ }^{\circ}\text{C}$. It is therefore important to cool the milk as quickly as possible after milking. Usually refrigerated milk is kept at a temperature of $4\text{ }^{\circ}\text{C}$. Bulk raw milk, when received from a chilling station in the factory should have a temperature below **7 - 8** $^{\circ}\text{C}$. The temperature of milk can be determined with a dairy thermometer; it is important to mix the milk well.

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1.8. PH test

This method is used screening. A rough estimate of pH may be obtained using paper strips impregnated with an indicator. Paper strips treated with Bromocresol purple and bromothymol blue can be used as screening tests for milk. Bromocresol purple indicator strips change from yellow to purple between pH 5.2 and 6.0, while bromothymol blue indicator papers change from straw yellow to blue green between pH 6.0 and 6.9.

1.8.1. Using pH meter

A pH meter depends on the potential difference between two electrodes when they are in contact with a test sample. One electrode called a reference electrode (a glass electrode) independent of the pH of the milk is connected to an electrode whose potential is proportional to the pH of the milk (a calomel electrode). The pH of the milk depends on the hydrogen ion concentration in the milk. A pH meter measures the current produced by the difference in potential between the two electrodes.

- First, the pH meter has to be prepared:
 - ✓ The pH meter should be kept in a dry atmosphere.
 - ✓ Before using a new glass electrode, or a glass electrode that has been stored for some time, soak the electrode in N/10 Hydrochloric acid for about 5 hours.
 - ✓ Care should be taken not to scratch glass electrodes against the sides of beakers or other hard surfaces during storage or testing.
 - ✓ The level of saturated potassium chloride in the calomel electrode should be checked before making pH measurements.
 - ✓ Crystals of potassium chloride should be present in the solution within the electrode.
 - ✓ The rubber stopper or cap on the filling arm of the calomel electrode should be removed before use.
- Then the pH meter should be standardized:
 - ✓ Rinse the electrodes with distilled water and wipe them gently with tissue or filter paper.
 - ✓ Use the control knob of the meter to set the temperature of the buffer used to standardize the meter.

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- ✓ Standardize the pH meter against a buffer solution of known pH. Use a buffer solution with a pH as close as possible to that of the test solution.
- ✓ Turn the range selector to the pH range covering the pH of the buffer control until the pointer of the meter reads the pH of the buffer.
- ✓ Set the range switch to zero.
- ✓ Before measuring the pH of the test sample, rinse the electrodes with distilled water and dry them.
- ✓ Set the temperature control knob to the temperature of the sample.
- ✓ Place the test sample in position and allow the electrodes to dip into the solution.
- ✓ Switch the range selector knob to the proper range and read the pH.
- ✓ Rinse the electrodes after use and keep the electrode tips in distilled water between tests.

Always follow the manufacturer's instruction for the particular instrument for interpreting the pH reading. Normal cow's milk has a pH of 6.5 to 6.8.

1.9. Clot on boiling test

Used to test for screening, rapid testing of increased acidity. The heating of milk in an advanced state of souring (acidity of more than 0.20%) or abnormal milk (colostrum or mastitis milk) will result in clotting.

The acidity of milk that gives a positive test is generally above 0.22% (as lactic acid) or has an abnormally high percentage of protein like colostrum milk. Such milk cannot stand the heat treatment in processing and is therefore not suitable for distributing as liquid milk or for processing. Such milk must therefore be rejected. Please take note that at high altitude milk boils at a lower temperature. This test is not very sensitive to slightly sour milk.

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1.10. Alcohol test

Used to test for screening and rapid assessment of acidity. Proteins in milk that has become sour (i.e. because of lactic acid formation) will coagulate when mixed with alcohol. It can be identified by mixing equal amounts (e.g. 2 ml) of milk and ethanol solution in test tube with the pipette and agitate by gentle movement and look for coagulation.

Milk containing more than 0.21% acid and milk that is abnormal (e.g. colostrum or mastitis milk) will not pass the test. This milk is not fit for further processing.

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Self –check-1.	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. List and discuss the method of milk test?(6pts)

Test II say true if the statement is correct say false if the statement is incorrect

1. Testing milk for organoleptic characteristics is also called sensory testing and uses the normal senses of sight, smell and taste in order to determine the overall quality. (2pts)
2. Alcohol test is Used to test for screening and rapid assessment of acidity (2pts)

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet 2. Evaluating organoleptic properties of final dairy products processing

2.1. Sight and smell test (organoleptic test)

The sight and smell test of milk products is done using normal senses of sight and smell in order to observe and record the overall quality. We get an instant result where and when it is carried out. If used correctly, it is very useful to do rapid screening of physical quality of milk products. It is the first and basic test for judgment of qualities of milk and various milk products. This test, of course, should be complemented by further laboratory tests. When milk is tested by taste to judge the quality of milk there is a risk of disease transmission, and this is not recommended from a health point of view.

Dairy product appearance can indicate either

- the presence of good qualities or
- Quality defects within the products.

In general, factors that may be evaluated by sight include:

- product color,
- the style of the product,
- condition of the package,
- attractiveness of product finish and workmanship,
- And overall appearance characteristics.

For practical example, a product may be presented for judging that is carelessly packaged and/or discolored in appearance. While these cues provide valuable information to the judge, caution must be exercised not to let the product appearance cues unduly influence the evaluator's taste and smell assessments of the given product.

Olfaction, or smelling, plays a critical role in the evaluation of dairy products. Using olfaction, important details about product quality can be determined. A large component of flavor is the specific odor property of the food; hence this attribute is critical in providing a thorough assessment of any dairy product. The sense of touch, including product texture and mouth feel, is also an important aspect of dairy judging for quality assessment.

- The feeling of rubberiness or stringiness of cheese,

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- the creamy or gritty mouth feel of ice cream and
- The “briny” defect in butter are all indications of product quality.

A judge’s fingers are also used on the external surface of some product samples to provide information on the relative springiness or hardness of a product.

Attributes of noise (sound) can also be used during dairy judging. For example, in Swiss cheese, quality can be evaluated by gently tapping the outside of the cheese with the fingers or a sampling device (cheese trier), which projects the relative size and/or distribution of “eyes” within the block of cheese.

Table 3. Organoleptic test results and interpretation for milk

Smell and/or tast	Interpretation
Souring	Lactose fermenting, acid producing bacteria
Bitter	Peptonising of milk by <i>Streptococcus liquefaciens</i>
Blue souring	unpleasant sweet and sour smell, thin and waterish appearance caused by bacterial activity and storage in a closed container without ventilation
Fruit aroma	<i>Pseudomonas</i> producing esters
Slimy milk	Indicates capsule forming bacteria, e.g. <i>Agrobacteria aerogenes</i> and <i>Alcaligenes viscosus</i>
Bubbles, coagulation and	Fermentation by yeast
Organoleptic test results and interpretation for cream	
Cooked taste	excessive heating
Tallow /oily	Fat oxidation
Rancidity	Fat hydrolysis
Barny smell	Poor ventilation
Bitter taste	Ingestion of bitter weeds
Cheesy taste	Increased activity of proteolytic bacteria
Barny smell	Poor ventilation



Self-cheek-2	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. Discuss about organoleptic test .(4pts)
2. Interpret test results of organoleptic test?(2pts)

Test II say true if the statement is correct say false if the statement is incorrect

1. The milk tasters should have good sense of sight, smell and taste of milk.
2. Equipment is required for organoleptic testing.
3. Since raw milk testing may pose risk of zoonotic disease to human.

Note: Satisfactory rating - 10 points Unsatisfactory - below 10 points
You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



InformationSheet-3. Recording and interpreting test results for dairy products

3.1. Recording and interpreting test results

Milk and milk products are sampled and tested for chemical, biological and physical characteristics. Milk in the upper part of the udder of a healthy cow generally contains lower microbial load than the opening of teats. Usually, total bacterial count in milk from a healthy is below 50,000 per ml. It may go up to several millions because of poor and unhygienic conditions during milking and milk handling and/or in milk from diseased cow.

Milk generally becomes sour 4-6 hours after keeping it at room temperature post milking. The onset of souring depends on quantity and quality of contamination and on milk temperature. The higher the number of lactic acid bacteria in milk, shorter the onset of souring (lowering of pH) and vice versa.

To conduct some tests, good laboratory infrastructure and facilities are required while for some other tests minimum laboratory infrastructure and facilities are required. To perform each test uniformly and perfectly by all laboratories a standard test protocols required to be followed by each laboratory.

This test protocol explains the procedure of conducting all the tests as mentioned in Table 6. For ease of understating of the test procedures, each step of conducting the tests is presented in the form of a flow chart.

Table 6. Methods of interpreting test result

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Table 6. Test protocol					
		Recommended by FSSA	Milk product quality relevance	Food safety relevance	Degree of difficulties to conduct
A. Physical tests					
1.	Sight and smell test/ Organoleptic Test	√	++		+
2.	Extraneous matter	√	++		+
3.	Determination of fat in milk product	√	++		++
4.	Determination of fat in milk product (Validated Method)	√	++		++
5.	Salt detection test	√	+		+
6.	Sugar detection test	√	+		+
7.	Maltodextrin detection test Enzymatic Method	√	+		+
8.	Starch detection test	√	+		+
9.	Urea detection test	√	+		+
10.	Neutralizers (Rosalic) detection test	√	+		+
11.	Method of Detection of Melamine in Milk and Milk Products	√		+	++++
12.	Detection of mineral oil in ghee (Holde's test)	√			+
13.	Butyrefractometer (BR) Reading for Detection of Vegetable Fat	√			++
14.	Ammonium Sulphate detection test	√			+
15.	Synthetic milk detection test				+
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16.	Glucose test (Validated Method)				+
17.	Test for presence of Boric acid and Borates (Validated Method)				+
B. Chemical tests					
18.	Determination of pH of milk product				+
19.	Determination of Temperature of Milk product	√	++	++	+
20.	Determination of Titratable Acidity as Lactic Acid	√	++		+
21.	Clot on boiling test	√	+		+
22.	Phosphatase test for pasteurized Milk	√	+		++
C. Food safety controls					
23.	Test for presence of Hydrogen Peroxide (H ₂ O ₂)	√	+		+
24.	Detection of Detergent residues	√		+	+



	Recommended by FSSA	Milk quality relevance	Food safety relevance	Degree of difficulties to conduct
25. Rapid test for detection of aflatoxin M1 in milk	√		+	++
26. Detection of heavy metal in milk	√		++	+
27. Detection of Insecticide & pesticide residues in milk	√		+	++++
28. Formalin test	√		+	+
D. Additional tests				
29. Test for presence of skimmed milk powder in natural milk		+		++
30. Test for Detection of Antibiotic Residues		+	+	+++
E. Microbiological test procedures				
31. Total Plate count or Standard plate Count	√	++	+	++
32. Yeast and Mould counts				++++
33. Coliform count	√	++	++	+++
34. <i>E. coli</i>	√	++	++	++++
35. <i>Staphylococcus aureus</i>	√		+++	++++
36. <i>Salmonella</i>	√			+++
37. <i>Listeria monocytogenes</i>	√		+++	++++
40. <i>Bacillus cereus</i>				++++
42. Milk ring test (MRT) for Brucellosis			+++	+
42. California Mastitis Test (CMT) for Mastitis			+++	+
43. MBR (Methylene Blue Dye Reduction) test for raw milk and pasteurized milk				+



Note

degree of relevance in regards to food safety:

Weak relevance= +

Medium relevance= ++

Strong relevance= +++

Degree of difficulties to conduct:

Least difficult=+

Difficult= ++

Fairly difficult= +++

Highly difficult= ++++

Milk and milk product tests are generally conducted by conventional methods that require different equipment, consumables, chemicals, glassware, etc. These conventional methods are labor intensive and time consuming. Despite the fact, most of the milk testing laboratories follow conventional methods as per unit cost of the test is relatively lower and it is perceived that results derived from the conventional methods are more accurate. In this protocol, all milk product tests are explained in conventional method under three different sub-heads as stated below.

- Reagents required
- Test procedure to be followed
- Interpretation of the results

In addition, some of the tests could be conducted by use of compact electronic machine (e.g. electronic milk analyzer) or by rapid test using ready-made kits. These tests are conducted following the manufacturers 'instructions. These tests give the results quickly.

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Self-cheek# 3.	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: fill the blank space with appropriate words.

1. Milk and milk products are sampled and tested for _____, _____and _____characteristic.(3pts).
2. _____explains the procedure of conducting all the tests.(1pt)

Test II say true if the statement is correct and false if the statement is incorrect

1. Total bacterial count in milk from a healthy is above 50,000 per ml.
2. To conduct some tests, good laboratory infrastructure and facilities are required.

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information Sheet-4. Documenting recommended specifications for physical, chemical and microbial properties

4.1. Acceptance levels

Recommended specifications for physical, chemical and microbial properties determines the acceptance levels of which the minimum requirements for the milk not to be completely rejected for the processing. If the milk meets these minimum requirements, the milk can be paid for according to the quantity and / or quality of the milk.

Table 7: Rejection and deduction levels for cow milk

Components	Rejection level	Deduction level	Unit
Fat	<3.0	3.0 - 3.2	%
SNF	<8.2	8.2 - 8.5	%
Total Solids	>12.0	10.0 - 12.0	%
Water or or or	<1.027	1.027 - 1.028	Density at 15 C
	>1.036	1.035 - 1.036	Density
	>-0.520	-0.520 to -0.525	°C freezing point
	>10	5 – 10	% excessive water
Preservatives	None	None	
Antibiotics	0.0006		i.u. / ml
Temperature			°C
PH.	<6.4	6.4 - 6.5	
Clot on boiling	Positive test	-	
Alcohol test	Positive test	-	
Titrateable acidity	0.20	0.18	% lactic acid
10 min Reassuring	0 and 1	2 and 3	Disc numbers
Methylene blue	<30	30 – 60	Minutes
Bacterial count	>750	500 – 750	(x1,000 CFU/ml)
Somatic cell count	>1,000	750 - 1,000	(x1,000 CFU/ml)



Self-check -4	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. Write the minimum requirements or recommended specifications for physical, chemical and microbial properties of milk?(5pts)

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



InformationSheet-5. Evaluating yield efficiency for process control parameters

5.1. Yield efficiency

Efficiency in the utilization of raw materials to optimize product yield is an important aspect of eco-efficiency and has the greatest scope for financial and environmental savings. Materials such as raw or pasteurized milk, cheese or whey, and components of milk such as fat, lactose and protein can be lost from the process and end up in the wastewater or solid waste stream. These losses are a waste of resources that could otherwise be recovered as products or co-products. They also contribute to the pollutant load of the waste water stream, resulting in increased treatment and disposal costs.

This section discusses opportunities to reduce waste in dairy manufacturing processes, hence helping to optimize yield and efficiently utilize raw materials. These initiatives can lead to the multiple benefits of reduced volumes of solid waste, reduced pollutant loads in wastewater and increased yields of saleable products.

5.1.1 Sources of product loss

Cheese yield is defined as the amount of cheese, expressed in kilograms, obtained from 100 kg of milk. It is a very important parameter: the higher the recovered percentage of solids, the greater is the amount of cheese obtained and therefore gains in economic terms. The definition of cheese yield, or how to express yield, is important in two main applications:

- Economic control of cheese making;
- Expressing the results of cheese making experiments.

Cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters.

Expression of actual yield can be rather meaningless because yield of cheese varies enormously for a number of reasons: variety of cheese and its typical composition; composition of milk (fat and casein/protein); composition of cheese (moisture, salt); losses of fat and curd during cheese making. It is usually useful to know what the yield of cheese of a typical constant composition should be from a lot or vat of milk.

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Cheese yield as affected by some parameters. For purposes of comparing the theoretical and actual yields. This theoretical yield is calculated from the composition of the milk by means of a yield formula. Yield can then be expressed as a percentage of theoretical yields. In some applications this can be termed cheese yield efficiency

5.1.2. Factors Affecting Cheese Yield

Cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters.

The casein fraction of milk protein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately affecting cheese quality and yield. Any factor affecting the casein content of raw milk has a potentially great impact on yield of cottage cheese. Manufacturer's profits depend on yield; thus, factors that decrease casein are costly.

Good quality milk, giving an optimal cheese yield and cheese quality, originate from healthy animals, has good flavor, has been cold stored for a limited amount of time and has a high protein content with the BB genotypes of s-lactoglobuline and κ -casein (bovine milk).

The milk should be low in somatic cell count, as proteases from somatic cells reduce the cheese yield.

The content of free fatty acids should be low, as free fatty acids bind Ca^{2+} and thereby reduce the coagulation properties of the milk, in addition free fatty acids contribute to the development of rancid flavor in cheese.

Milk used for cheese making must be of good microbiological quality, pathogenic bacteria should be absent, psychotropic bacteria have heat resistant lipases and proteases which may reduce yield, but may also cause undesirable flavors in the ripened cheese.

Cheese moisture. A 1% increase in Cheddar cheese moisture causes about 1.8% increase in cheese yield, partly because more moisture means more whey solids and

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salt are recovered in the cheese (e.g, given 90 kg cheese/1000 kg milk, a moisture adjustment to 36% would result in 91.6 kg cheese/1000 kg milk)

Cheese salt. An extra 0.1% salt means an extra 0.14% yield of Cheddar cheese if the moisture content is increased accordingly. Increasing time and temperature of milk pasteurization increases cheese moisture retention and the recovery of whey proteins and soluble solids. There doesn't seem to be any consensus on how much is desirable but it's safe to say that it depends on the type of cheese and the quality standards of the manufacturer.

5.1.3. Process control parameters

- Careless cutting.
- Heating too fast at early stages of cooking
- Salting too soon after milling of Cheddar allows rapid salt uptake which in turn causes rapid syneresis and increased solubility of casein. Yield is, therefore, reduced by losses of protein, fat and soluble solids.
- High temperatures during pressing cause loss of fat.
- Photolytic cultures or coagulating enzymes cause protein losses before and after cutting.
- Washing removes soluble solids.
- Working as in Mozzarella removes fat and soluble solids. Loss of soluble solids is minimized by equilibration of the wash water with the cheese moisture.

5.2. Principles of Yield Optimization

With respect to yield the cheese maker's objectives are to: Obtain highest MNFS (moisture in non-fat substance) consistent with good quality to maximize moisture and the recovery of whey solids. Standardize milk to obtain maximum value for milk components consistent with good quality (eg., adjust P/F to maximize cost efficiency). Minimize losses of fat and casein in the whey.

5.2.1. Yield Control

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It is absolutely vital to be able to measure and maximize yield efficiency. This means maximizing the return (or minimizing the loss in the case of lactose) from all milk components entering the plant. This includes obtaining maximum returns for whey non-fat-solids, whey cream and cream skimmed during standardization. In general the highest return for all milk components is obtained by keeping them in the cheese, but this may not always be the case.

5.2.2. Recovery of Milk Components

Yield efficiency can be determined by monitoring recovery of milk components and losses in the whey. By keeping accurate records of all incoming milk components and their distribution between cream, cheese, whey cream and defatted whey it is possible to determine the plant mass balance.

5.3. Yield Prediction

- Yield Prediction Provide a target against which to judge actual yields and determine mass balance within the plant.
- Flag errors in measurement: e.g. weights of milk or improper standardization etc.
- Early signal of high or low moisture content which allows adjustment on the following vats. This can be met by rapid moisture tests (microwave) which is sufficiently accurate for this purpose

The Van Slyke and Price Formula

$$Yield = \frac{(0.93F + C - 0.1)(1.09)}{1 - M} = 9.945\%$$

Van Slyke formula is based on the premise that yield is proportional to the recovery of total solids (fat, protein, other solids) and the moisture content of the cheese.

F = Fat content of milk (3.6 kg/100 kg)

C = Casein content of milk (2.5 kg/100 kg)

0.1 = Casein lost in whey due to hydrolysis of -casein and fines losses

1.09 = a factor which accounts for other solids included in the cheese; this represents calcium phosphate/citrate salts associated with the casein and whey solids

M = moisture fraction (0.37)

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This formula has several important limitations: First, it's difficult to measure casein. Many plants use total protein in the predictive formula and multiple by a factor to estimate casein. The classical procedure for casein determination is Rowland Fractionation which is too involved for most cheese plants. A second difficulty is that the formula fails to consider important variables such as variation in salt content and whey solids. Third difficulty is that the equation is quite specific to Cheddar.

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Self-check - 5.	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

- 1. What are the factors Affecting Cheese Yield?(5pts)
List the Principles of Yield Optimization (4pts).

Test II: choose the best answer for the following questions

- 1. The amount of cheese, expressed in kilograms, obtained from 100 kg of milk is known as_____?(3pts)
 - A. control parameter
 - B. Yield Prediction
 - C. Yield efficiency
 - D. all
- 2. Yield efficiency can be determined by_____?(3pts)
 - a. By monitoring recovery of milk components and losses in the whey.
 - b. By keeping accurate records of all incoming milk components and their distribution.
 - c. by determining the plant mass balance.
 - d. all
- 3. what is importance of Yield Control?(3pts)
 - a. to measure and maximize yield efficiency.
 - b. to minimize the loss in the case of lactose from all milk components.
 - C. obtained maximum returns for whey and non-fat-solids
 - d. all are answers

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information sheet- 6. Referring specifications against test data

6.1. Standards of Milk

The standards are used as a reference and helps to Carried out testing and interpreting results of milk products to make adjustments to the dairy products processing processes. To have further process the quality of milk and milk products first tested and referenced with the standards to make adjustment and control the process.

The dairy sector includes food such as liquid milk, milk powders, cheese, butter, and yogurt, as well as ice cream. Several factors including:

- genetics, and breed of animal,
- environment,
- stages of lactation,
- parity, and nutrition,

Together determine the final composition of milk and milk products. Milk and dairy products are significant sources of protein, essential minerals (calcium, potassium, magnesium, phosphorous, sodium, iodine) and several vitamins, (the fat-soluble vitamins A, D, E, K, and B1, B3, B6, B12). In a Western diet, dairy products provide between 40 and 70% of the recommended daily calcium intake. Cow's milk consists of about 87% water, and 12–13% total solids.

The solids consist of fat ~4% and solids-not-fat (SNF) ~9%, such as proteins, lactose, and various minerals and vitamins. Milk proteins consist of whey and caseins; caseins have four different species (α_{S1} , α_{S2} , β , and κ -caseins) which are separate molecules, but they do possess similarity in structure and they comprise around 80% of total milk protein. The milk proteins contain the nine essential amino acids required by humans, making it an important human food. The caseins are easily digested, while the whey proteins are relatively less digestible in the intestine.

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Table 8. Average composition of milk and milk powders.

Content	Cow's milk %	Skim milk powder (SMP) %	Whole milk powder (WMP) %	Acid whey powder (WP) %
Moisture	85.5–89.5	3.0–4.0	2.0–4.5*	3.5–5.0
Fat	2.5–6.0	0.6–1.5	26.0–42.0	1.0–1.5
Protein	2.9–5.0	34.0–37.00	24.5–27	11.0–14.5
Lactose	3.6–5.5	49.5–52.0	36.0–38.5	63.0–75.0
Minerals (ash)	0.8–0.9	8.2–8.6	5.5–6.5	8.2–8.8

The moisture content does not include water of crystallization of the lactose, the milk solids-not-fat content includes the water of crystallization of the lactose.

Table 9. Quality analytical tests for raw milk.

Quality tests	Acceptable limits	Standards	Reference number
Acidity (Titratable)	≤0.18%	ISO 6091:2010	[19]
Antibiotic residues	Absent/0.1 g	ISO 26844:2006c	[20]
Freezing point (added water)	–0.54°C	ISO 5764:2009	[21]
Fat	0.8%	ISO 1736:2008	[22]
Protein	34%	ISO 8968–1/2:2014 and ISO 14891:2002	[23, 24]
Lactose	>4.2%	ISO 22662:2007	[25]

The ISO standards catalog ISO/TC34/SC5 lists all milk and milk products standards, while other standard sets include, microbiology of the food chain, microbiological quality of milk, etc. The bacterial quality of the milk is also measured and these specify tests are outlined later.

The titratable acid test measures the acidity of the milk. Both titratable acidity (TA) and pH are measures of acid. TA is a more reliable indicator because relative to pH



measurement, it is more sensitive to small changes in milk acidity, especially important in cheese making. The acidity of milk is of two types; natural acidity due to citrates and phosphates present in the milk and dissolved CO₂ during the processing of milking.

The second is the developed acidity due to lactic acid produced by bacteria using the lactose in the milk as a nutrient, converting it to lactic acid. The acidity of milk measures the total acidity (natural acidity of milk and developed acidity).

The International Standard Method for titratable acid is ISO 6091:2010. Titratable acidity is a measure of the buffering of milk between pH 6.6 and 8.3 (phenolphthalein endpoint). The appearance of a faint pink color, which signals the endpoint and the number of ml of NaOH used to reach the endpoint, is recorded. This value is called the “titer,” titratable acidity is reported as percent lactic acid and is dependent on the volume of sample. As this test is dependent on the analyst reading eye measurement of the color change, it is prone to human error causing incorrect and unpredictable recording of results.

6.2. Standards of cream

Cream is not a definite specific substance. It contains all the milk constituents but in varying proportions. Cream for sale to consumers is produced with different fat contents. Cream of lower fat content, 10 – 18%, is often referred to as half cream or coffee cream, it is increasingly used for desserts and in cooking. Cream with a higher fat content, typically 35 – 40 %, is usually considerably thicker. It can be whipped into a thick froth and is therefore referred to as “whipping cream”. The milk fat in cream may vary from 10 to 75 per cent.

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Table 10: Chemical composition of cream

Constituents	Percentage
Water	45.45 - 68.2%
Fat	25 - 60%
Protein	1.69 - 2.54%
Lactose	2.47 - 3.71%
Ash	0.37 - 0.56%
Total solids	31.8 -54.55%
Solids not fat	4.55 - 6.80%

6.2.1. Standardization of Cream

This refers to the adjustment of the fat level in cream to the desired percentage, confirming to standard requirements. The fat percentage in cream is adjusted to the prescribed level by the addition of skim milk or cream. Desired level of fat in cream for butter making is 33 to 40 per cent. Standardization to both higher and lower level leads to higher fat loss in butter milk. Reduction of fat by adding water should be avoided as it interferes with ripening of cream and also results in butter with 'flat' or 'washed off' flavor.

6.3. Butter

Butter is a fat rich dairy product, generally made from cream by churning and working. It contains 80% fat, which is partly crystallized. Butter making is one of the oldest forms of preserving the fat component of milk. Its manufacture dates back to some of the earliest historical records, and reference has been made to the use of butter in sacrificial worship, for medicinal and cosmetic purposes, and as a human food long before the Christian era. Butter can be produced from the milk of cow, buffalo, camel, goat, ewe and mares. Fat is separated from milk in the form of cream using cream separator. The cream can be either purchased from a fluid milk dairy or separated from whole milk by the butter manufacturer. The cream should be sweet (pH greater than 6.6), not rancid, not oxidized, and free from off flavors. The cream is



pasteurized at a temperature of 80°C or more to destroy enzymes and micro-organisms.

6.3.1. Composition of Butter

Butter is principally composed of milk fat, moisture, salt and curd. It also contains small amount of lactose, acids, phospholipids, air, microorganisms, enzymes and vitamins. The proportion of principal constituents in butter is largely controlled by the method of manufacture and this in turn is chiefly regulated to conform to the standards of butter.

Table 11: Composition of butter

Constituents	Quantity (% w/w)
Fat	80-83
Moisture	15.5-16.0
Salt	*0-3

Table 12: Microbiological standards of Butter

Total Plate Count Max	50,000/g
Coliform Count	50/g
E. Coli	Absent/g
Salmonella	Absent /25g
Staph aureus	50/g
Yeast and mold count	50/g
Listeria Mono cytogenes	Absent/g
Sampling Guidelines	100g Sample and -18°C or lower storage temperature

6.4. Ghee

Ghee means the pure heat clarified fat derived solely from milk or curd or from desi (cooking) butter or from cream to which no coloring matter or preservative has been added. Ghee essentially consists of 99 to 99.5% milk fat.



Table 13: Chemical composition of ghee

Constituents	Ghee
Fat(%)	99-99.5
Moisture	<0.5
Carotene(mg/g)	3.2-7.41
Vitamin(IU/g)	19-34
Cholesterol (mg/100 g)	302-363
Tocopherol(mg/g)	26-48
Free Fatty Acid	2.8

6.5. Cheese

The word 'cheese' is derived from the Old English 'case' which in turn was derived from the Latin 'causes' which means correct or perfect thing. Cheese may be defined 'as the curd of milk separated from the whey and pressed into a solid mass'. This definition of cheese is satisfactory but too limited and vague from a technical standpoint. Therefore, a relatively complete definition is as follows:

Cheese is the curd or substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms, from which part of the moisture has been removed by cutting, warming and pressing, which has been shaped in mold and then ripened (also unripen) by holding for some time at suitable temperatures and humidity.

There are probably only about 18 types of natural cheeses. These are: Cheddar, Gouda, Edam, Swiss, Brick, Hervey, Camembert, Limburger, Parmesan, Provolone, Romano, Roquefort, Sapsago, Cottage, Neufchatel, Trappers, Cream and Whey cheeses.



6.4.1. Certain standards for cheese

Such a grouping, though informative, is imperfect and incomplete. These can also be classified on the basis of their rheology, and according to the manner of ripening as shown below:

- 1) Very hard (grating) - Moisture < 35% on matured cheese and ripened by bacteria,
e.g. Parmesan, Romano.
- 2) Hard - Moisture < 40%
 - a) Ripened by bacteria, without eyes: Cheddar
 - b) Ripened by bacteria, with eyes: Swiss
- 3) Semi-hard - Moisture 40-47%
 - a) Ripened principally by bacteria: Brick
 - b) Ripened by bacteria and surface microorganisms: Limburger
 - c) Ripened principally by blue mould:
 - i) External – Camembert
 - ii) Internal – Gorgonzola, Blue, Roquefort.
- 4) Soft - Moisture > 47%
 - a) Unripen – Cottage
 - b) Ripened – Neufchatel

Table 14: Legal standards of cheese

Type of cheese	Moisture, maximum	Milk Fat (on dry basis), minimum
Hard pressed cheese	39.0%	48.0%
Semi hard cheese	45.0%	40.0%
Semi soft cheese	52.0%	45.0%
Soft cheese	80.0%	20.0%
Extra hard cheese	36.0%	32.0%
Mozzarella cheese	60.0%	35.0%
Pizza cheese	54.0%	35.0%



6.6. Ice Cream

According to Food Safety and Standards Regulation ice cream or softy ice cream means the product obtained by freezing a pasteurized mix prepared from milk and/or other products derived from milk with or without the addition of nutritive sweetening agents. The product shall conform to the requirements given in Table 8.

Table 15: Food Safety and Standards for Ice cream

Requirement	Ice Cream	Medium Fat Ice Cream	Low Fat Ice Cream
Total Solid	Not less than 36.0 percent	Not less than 30.0 percent	Not less than 26.0 percent
Wt/Vol (g/l)	Not less than 525	Not less than 475	Not less than 475
Milk Fat	Not less than 10.0 percent	More than 2.5 percent but less than 10.0 percent	Not more than 2.5 percent
Milk Protein (Nx6.38)	Not less than 3.5 percent	Not less than 3.5 percent	Not less than 3.0 percent

i.e. The standards discussed in the tables above for dairy products are the reference to compare and determine whether the acceptance or rejection of dairy food for processing.



Self-cheek -6.	Written test
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Name.....ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. What are the reference points used to test data?(5pts)
2. Determine the standards of milk composition (4pts).
3. What is cheese and what are the cheese types list at least 4 types of cheese.(5pts)
4. Determine Food Safety and Standards for Ice cream.(3pts)

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



Information Sheet -7. Implementing changes to cheese making process

7.1. Chemical and physical changes

Cheese ripening is basically about the breakdown of proteins, lipids and carbohydrates (acids and sugars) which releases flavour compounds and modifies cheese texture. The biochemical and biophysical processes involved have only partly been elucidated. Here we include only a few practical principles of ripening.

7.2. General Principles

- Ripening varies from nil for fresh cheese to 5 years for some hard ripened cheese. Like a good wine, a good aged cheese should get better and better with age.
- Ripening processes are broadly classified as interior and surface ripened.
- Cheese which depend mainly on interior ripening (most hard ripened cheese such as Cheddar and Italian types) may be ripened with rind formation or may be film wrapped before curing.
- Cheese which depend mainly on surface ripening include smear ripened and mould ripened
- In the broadest terms there are three sources of cheese flavour:
- Flavours present in the original cheese milk, such as natural butter fat flavour and feed flavour.
- Breakdown products of milk proteins, fats and sugars which are released by microbial enzymes, enzymes endogenous to milk, and enzyme additives.
- Metabolites of starter bacteria and other microorganisms. These include products from catabolism of proteins, fats and sugars.

Flavour and texture development are strongly dependent on:

- pH profile
- Composition
- Salting
- Temperature
- Humidity

As a general rule factors which increase the rate of ripening increase the risk of off flavour development, and reduce the period of time when the cheese is saleable.

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7.2.1. Protein Breakdown (Proteolysis)

Natural degradation of protein is called 'putrefaction' and results in 'rotten potato' type odors, especially if high quality proteins such as animal proteins are involved. That's because animal proteins contain the essential sulfur amino acids. These 'putrefactive' components are also the stuff of which good flavours are made. Protein degradation during cheese curing is a directed process resulting in protein fragments with desirable flavours.

- Some off flavours associated with undesirable or excessive protein breakdown in cheese are bitter, stringy, putrid and brothy.
- Protein breakdown causes shorter body which is less rubbery, less elastic, and more meltable. For example, flavour and texture development in Cheddar are mainly dependent on protein breakdown and much less dependent on fat breakdown.
- Protein breakdown involves three general types of processes:
- Proteases break proteins into smaller peptides, some of which are flavour compounds. For example, bitter and brothy flavored peptides are well known to occur in cheese.
- Peptidases further break down peptides to amino acids.
- Further catabolism of amino acids by cheese microorganisms produces aldehydes, alcohols, carboxylic acids and sulfur compounds, many of which are flavorful.
- The amino acid, tyrosine, forms crystals in aged cheese such as Parmagiano regiano, which are readily detected on the palate.

7.2.2. Fat Breakdown (Lipolysis)

Dairy fat is a wonderfully rich source of flavours, because it contains an extremely diverse selection of fatty acids. In particular, butter fat is the only natural fat which is rich in short chain fatty acids. Butyric acid for example is a potent flavour compound. As with all potent flavours the trick is to add just the right amounts in balance with other flavours. Here are a few principles:

- Dairy fat without any ripening during cheese making is an important contributor to cheese flavour and texture:

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- Fresh dairy fat has the well-known 'buttery' flavour associated with extremely low levels of free fatty acids.
- Fat also acts as a flavour reservoir, so hydrophobic (fat soluble) flavours derived from protein breakdown are stored in the fat and released during mastication in the mouth.
- Finally, fat is an important component of cheese softening and melting.
- The fat derived flavours associated with cheese ripening result from the release of fatty acids by lipolysis and further modification of fatty acids by microorganisms to other compounds.
- Varieties traditionally made from goats' milk have higher levels of lipolysis.
- Blue moulds are generally quite impolitic

7.2.3. Lactose

Milk contains no starch or fiber or any sugar other than lactose so all carbohydrate compounds in cheese are derived from lactose or produced by microorganisms. Relative to fat and protein lactose contributions to flavour are minimal. Here's a few principles:

- At Day 1 following cheese manufacture most of the milk sugar has been removed in the whey by or by fermentation that is converted to lactic acid by the cultures.
- Residual lactose depends on the type of cheese and other factors. For examples:
- High salt in the moisture phase of Cheddar slows lactose metabolism so lactose content is .3 to .7%% at one day after manufacture and slowly declines to less than 0.1%.
- Residual lactose in Camembert cheese is used by *Penicillium camembert* so it decreases quickly, especially on the surface, when the mould begins to grow.
- In well drained cheese such as Swiss types, lactose is completely used up in a few hours.
- In washed cheese varieties, lactose not leached by washing is quickly used up by the culture, especially for Dutch type cheese where salting is delayed. In Colby, early vat salting reduces the rate of utilization of residual lactose.

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- Many organisms, including yeasts and moulds in mould and smear ripened cheeses utilize lactate and produce various flavorful compounds.
- Calcium salts of lactic acid may form white precipitates on the surface of aged cheese.

7.3. Principal Ripening Agents

7.3.1. Milk Enzymes

Plasmin: A milk protease which survives pasteurization and breaks down caseins during cheese ripening.

- Particularly important in Swiss type cheese.
- Inhibited by Beta-lactoglobuline, so it has minimal activity in cheese made from ultra-filtered milk.
 - ✓ Lipoprotein lipase is the principal milk lipase
 - ✓ Inactivated by low heat treatment but is important to flavour development in raw milk cheese

7.3.2. Milk Coagulant

Each milk coagulant has its own proteolytic profile (see section on coagulants).

- Purified extracts produce more consistent flavours but lack character.
- For aged cheese no enzyme other than calf rennet and recombinant calf rennet has proven fully acceptable.
- Rennet and recombinant rennet actively break down alpha-casein but do not break down beta-casein in cheese.

7.3.3. Lactic Cultures

During the early days and weeks of ripening, LAB numbers decrease while the numbers of nonstarter bacteria decrease. For example, in Cheddar cheese, LAB counts reach a maximum (up to 500 million per gram) within 3-4 days and then decrease to about 20 million at 4 weeks. However, the dying cells release enzymes which continue to ripen the cheese.

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Lactic cultures contribute to proteolysis flavours but are minimally impolitic
Hetero fermentative cultures ferment citrate as well as lactose and contribute both flavour (diacetyl) and carbon dioxide for small eye development

7.3.4. Secondary Cultures

In Swiss types, carbon dioxide production by Propionibacterium is encouraged by exposure to 200C for about 3 weeks after brining and drying off in the cold room.

- For smear ripened cheese, Brevibacterium linens , coryneform bacteria, and yeasts are encouraged by high humidity (90-95%) and washing to discourage moulds
- Penicillium sp. for Camembert, Brie and Blue types require 85-90% humidity and air circulation to provide oxygen

7.3.5. Non-starter Microorganisms

Microorganisms present in the milk due to environmental contamination are important contributors to milk ripening. Some important facts are:

Bulk cooling and storage of raw milk selects for cold tolerant (psychrotrophic) bacteria.

Heat treatment selects for thermal stable spore forming bacteria

Non-starter bacteria commonly present in heat treat Cheddar include Lactobacillus sp. and Pediococci sp.

Many other bacteria and yeasts may be present and may or not grow depending on complex symbiotic relationships with other bacteria.

Heat treat is really a process of standardizing the none tarter microorganisms, namely, eliminate proteolytic Psychrotrophic bacteria but retain a range of useful ripening microbial agents.

Non-starter bacteria in cheese milk can be reduced by microfiltration.

7.3.6. Added Ripening Agents

Addition of lipases as noted earlier is common for Italian and other cheese varieties.

The principal areas of continuing development are:

- Accelerated ripening agents for all ripened cheese, especially Cheddar
- Ripening agents for low fat cheese, again especially Cheddar.

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The principal approaches are:

- Direct addition of single enzymes of dairy or non-dairy sources
- Enzyme cocktails which are mixtures of proteases and lipases. Other than in the preparation of enzyme modified cheese pastes, enzyme cocktails have had limited commercial success.
- Enzyme capsules which release trapped enzymes during ripening.
- Attenuated (freeze shocked or heat shocked) proteolytic cultures
- Genetically modified cultures hold lots of promise for future success.
- Culture adjuncts such as *Lactobacillus helveticus* in Cheddar cheese hold much promise to replace the normal diverse micro flora of raw milk.

7.4. Cheese Composition for Optimal Curing

Cheese composition is critical to yield optimization, and both flavour and texture development. This section gives some detail on several critical composition parameters, with special reference to Cheddar cheese.

- MNFS(moisture non fat solid)
- Moisture: higher moisture means faster ripening which means more potential for off flavours and over ripening.
- water activity (a_w) decreases with age because ripening results in many soluble breakdown products of acids, sugars, proteins and lipids
- fresh Cheddar $a_w = 0.98$ which is conducive to most bacteria
- aged Cheddar a_w as low as 0.88 which is too low for most bacteria
- MNFS is a better index of cheese ripening potential than % moisture
- Optimum MNFS depends on expected date of maturity and curing temperatures:
- examples for Cheddar: 100C, 6-7 months MNFS = 53%
- 100C, 3-4 months MNFS = 56%
- MNFS is controlled mainly by pH at dipping and cooking treatments. Subsequent curd treatment such as cheddaring and salting also influence MNFS
- MNFS is also influenced by FDM. Other conditions being kept constant, MNFS increases with increasing FDM, because fat inhibits syneresis.
- S/M

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Determines rate of acid development during pressing and early curing and, therefore, influences the minimum pH

- Affects bacterial profile, eg., high S/M will discourage contaminating bacteria such as coliforms.
- Critical to rate of proteolysis and the type of protein derived flavours
- Acceptable range is broad (3.6 - 6.0), fortunately because S/M varies widely even within a single cheese.
- Salt uptake is affected by quantity of added salt, size of curds, moisture **content of curds, and acidity**

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Self check -7	Written exam
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Short Answer Question

1. What are the factors Affecting Cheese Yield?(5pts)
2. List the Principles of Yield Optimization (4pts).

Test II: choose the best answers for the following questions

1. Cheese ripening is basically about the breakdown of proteins, lipids and carbohydrates which releases flavour compounds and modifies cheese texture.

1.The amount of cheese, expressed in kilograms, obtained from 100 kg of milk is known as_____?(3pts)

- A. control parameter
- B. Yield Prediction
- C. Yield efficiency
- D. all

2. Yield efficiency can be determined by_____?(3pts)

- a. By monitoring recovery of milk components and losses in the whey.
- b. By keeping accurate records of all incoming milk components and their distribution.
- c. by determining the plant mass balance.
- d. all
- d. all are answers

Note: Satisfactory rating - 18 points Unsatisfactory - below 18 points

You can ask you teacher for the copy of the correct answers.

Score = _____
Rating: _____



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