MILK MICROBIOLOGY

Microbiology

What is Milk Microbiology?

Basically it’s the study of Milk and its microorganism.

Microbiology?

Is the study of Microorganisms; under this are bacteria, virus, protozoal parasites, and fungi.

What is Milk?

- It is a white liquid produced by mammary gland of animals. It is the primary nutrition of the young before they are able to digest more complex food. Throughout the world there are 6 millions consumers in the world.
- Milk is sterile at secretion in the udder but is contaminated by bacteria even before it leaves the udder. Further infection of the milk by microorganisms can take place during milking, handling, storage, and other pre-processing activities.
- Raw milk - The lacteal secretion, practically free from colostrums obtained by the complete milking of one or more healthy cows (PMO).
- “Consumer Milk” products:
- - Homogenized milk: ≥3.25% fat
- - Reduced fat milk: 2% fat
- - Low fat milk: 1% fat
- - Fat-free milk: skim milk, <0.5% fat
- (all with 8.25% solids-non-fat)

Introduction

Fermented milk or dairy products have been part of human diet since ancient times. Various fermented products are made by different strains. Lactic acid fermentation is performed most often by lactic acid bacteria. Due to their abundance in nature, including mucosal surfaces of the human body, and their use in fermented foods they are labeled as GRAS (generally recognized as safe). The main genera that belong to the lactic acid bacteria group are: *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Streptococcus*.

These bacteria ferment the carbohydrates in milk, the major one being lactose, to lactic acid and some other products. The acid precipitates the proteins in the milk and that is why fermented products are usually of thicker consistency than milk. The high acidity and low pH hinders the growth of other bacteria, including pathogens. Some lactic acid bacteria can produce agents with antimicrobial properties. Since milk is rich in many nutrients such as protein, calcium, phosphorus, and B vitamins dairy products are an excellent food

Physical and Chemical Properties of MILK

- It is a white emulsion or colloid of butter fat globules
- Has yellow-orange carotene imparts the creamy yellow color to the glass.
- Contains 30-35% grams of protein per liter of which about 80% is arrange in casein micelles
- Different carbohydrates such as: lactose, glucose, galactose, and other oligosaccharides
- Vitamins A, B6, B12, C, D, K, E, thiamine, niacin, biotin, riboflavin, folates, and pantothenic acid are present

Origin of microorganism in milk

- **Commensal micro flora-** teat skin, epithelial lining of the teat canal, duct that conveys the milk from the mammary gland to the teat orifice.

- **Environmental contamination-** soil, water equipment, dairy farm area are reservoir for many food borne pathogens

YOGURT

a semisolid sourish food prepared from milk fermented by added bacteria, often sweetened and flavored.

YOGURT

- Mixed starter culture – *S. Thermophilus* and *lactobacillus delbrueckii or lactobacillus bulgaricus.*
- Ratio 1:1
- Fermentation: lactose content of milk to yield lactic acid, CO2, acetic acid, diacetyl and acetaldehyde
- Ph reduce: around 6.5 to 4.5 due to the production of organic acids
- Initially *streptococcus thermophilus* ferments the lactose
- Lactobacillus bulgaricus, which is more acid tolerant, continues to ferment the remaining lactose.

BENEFITS of Yogurt

- - Easier digestibility,
- - The ingested organisms enhance bioavailability of nutrients
- - Ensure gastrointestinal balance,
- - Promoting colon health
- - Accelerates the healing of gastrointestinal tract disorder
- - Reduction in cholesterol level.

Changes in milk by microorganism

- Gas production: Fermentation occurs at faster rate, then raw milk present a foamy layer on the upper surface Air bubbles becomes entrapped and gas becomes saturated throughout the body of the milk. *Colliforms, clostridium* and *bacillus* species.
- Roppiness or sliminess: Milk viscosity is increased, rope like structure is formed

Changes in milk by microorganism

Change in the color of milk

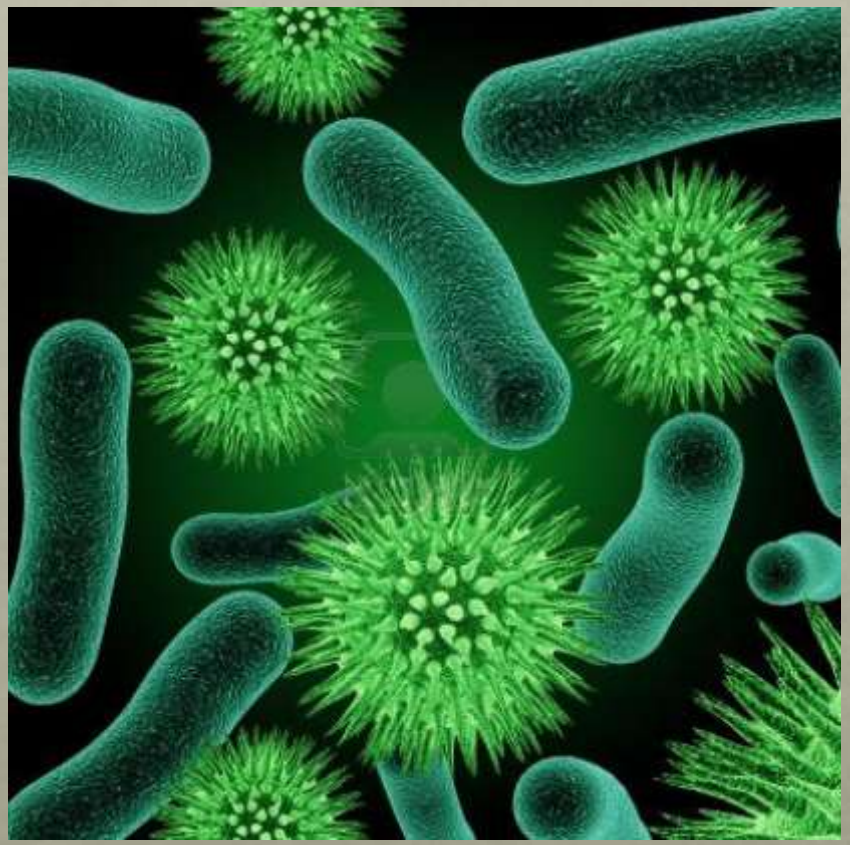
- Blue color – pseudomonas synciani
- Red color – brubibacterium erythrogenes, sarcinia marcense
- Yellow color – pseudomonas synxantha
- Brown color – pseudomonas putrificians
- Green color – pseudomonas aurogenosa

Change in the Flavor of Milk

- *Sour Flavour*:
  It is due to acidic changes in the milks:
- - Clean: Low contents of acids, *Streptococcus lactis*
- - Aromatic: *streptococci* and aroma- forming *Leuconostoc* sp. , moderated type of acidic components.
- - Sharp: *coliform* bacteria, *clostridium* species, volatile fatty acids, high acidic contents
- *Bitter Flavour*:
  It is due to alkaline changes in the milk.*Potato-like Flavour*:
  Pseudomonas mucidolense
- *Fishiness:*
  Acromian hydrophila, It is due to formation of tri-methyl amine

IMPORTANCE OF BACTERIA IN MILK

1. Bacteria – Are microscopic, unicellular, occurs in the form of spherical, cylindrical or spiral cells; Size 1-5m. Sore forming bacteria produce trouble in dairy industry because of their resistance to pasteurization & sanitization produces. Greater the bacteriological count in milk, the lower is its bacteriological quality. Pasteurized milk should have a SPC (Standard Plate Count)/ml (org) not exceeding 30,000.



IMPORTANCE OF BACTERIA IN MILK

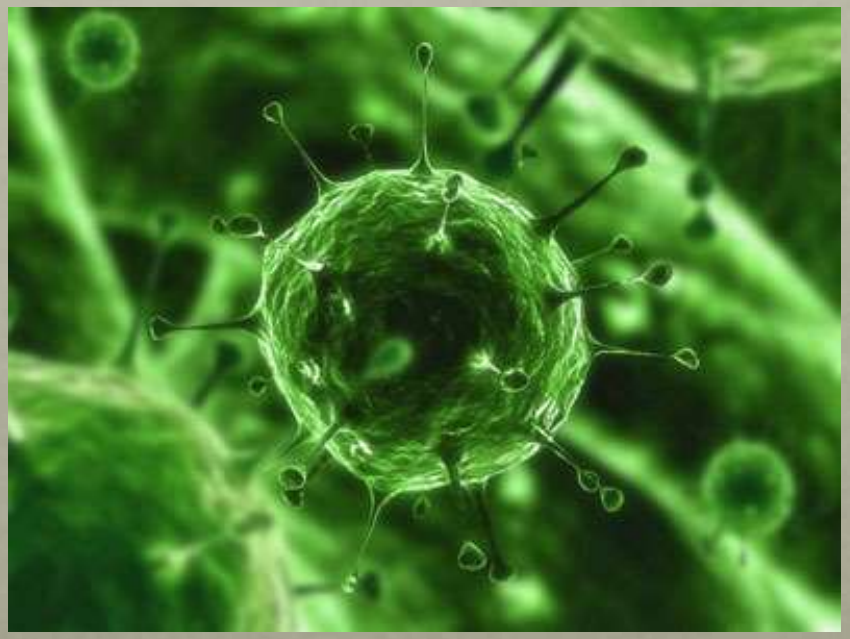
2. Moulds/ Molds – Multi-Cellular; in maturity are as Mycelium. Used in cheese making which is responsible for defect in butter and other milk products. Spores are destroyed by pasteurization. It should also be noted that moulds, mainly of species of Aspergillus , Fusarium , and Penicillium can grow in milk and dairy products.

IMPORTANCE OF BACTERIA IN MILK

3. Yeast – Unicellular; Larger than Bacteria. Destroyed during pasteurization.

IMPORTANCE OF BACTERIA IN MILK

4. Viruses – Are ultra-microscopic forms of like can be destroyed by pasteurization or higher heat treatment.



GROWTH OF MICROORGANISM

Bacteria multiply during production and holding of milk, depending on storage time and conditions. The changes take place in the physico-chemical properties of milk are result of the activities of the individual microbial cells during their period of growth and reproduction or of substances produced during such activity.

GROWTH OF MICROORGANISM

Stages of growth :

- i. Initial stationary phase
- ii. Lag phase (Phase of adjustment)
- iii. Accelerated growth phase (log phase)
- iv. Maximum stationary phase
- v. Phase of accelerated death.

Products of Microbial Growth :

- i. Enzymesii. Decomposition products (fats, proteins, sugars).
- iii. Pigments
- iv. Toxins
- v. Miscellaneous changes.

GROWTH OF MICROORGANISM

- **Destruction of Micro-organisms :** May be done by following means.
- i. Heat – Most widely used. Pasteurization & sterilization.
- ii. Ionizing radiation – Such as ultraviolet rays etc.
- iii. High frequency sound waves – Supersonic and ultrasonic.
- iv. Electricity – Microbes are destroyed actually by heat generated.
- v. Pressure – Should be about 600 times greater than atmospheric pressure.
- vi. Chemicals – Includes acids, alkalis, hydrogen peroxide, halogens etc.

Factors Influencing Growth :

- i. Food supply – Milk and its products are good food source, provides all food requirements.
- ii. Moisture – Milk contains adequate moisture to development.
- iii. Air – Supplies O2 to aerobic bacteria and moulds.
- iv. Acidity or pH – Preferably range 5.6 to 7.5.
- v. Preservatives – Check growth depending upon concentration.
- vi. Light – More or less harmful.
- vii. Concentration – High sucrose or salt content check growth.
- viii. Temperature – Important means for controlling growth. According to their optimum growth temperature, bacteria can be classified into :
- ix. Psychotropic – can grow at refrigeration temp. 5-7^{0}C.
- x. Mesophilic – can grow at temp. 20-40^{0}C.
- xi. Thermophilic – can grow at temp. above 50^{0}C.

Results of Microbial Growth in Milk :

- i. Souring:- Most common, due to transformation of lactose into lactic acid & other volatile acids & compounds, principally by lactic acid bacteria.
- ii. Souring & gassiness:- Caused by coil group, indicates contamination of milk and its products.
- iii. Aroma production:- Due to production of desirable flavour compounds s.a. diacetly.
- iv. Proteoloysis:- Protein decomposition leading to unpleasent odour.
- v. Ropiness:- Long threads of milk are formed while pouring. Mainly Alkaligenous viscus.
- vi. Sweet curdling:- Due to production of a remain like enzyme curdles milk without souring.

DISEASES Outbreaks ASSOCIATED with MILK PRODUCTS

ORGANISM	*DISEASE*	*SYMTOMS*	*SOURCE*
Campylobacter jejuni	Gastroenteritis	Diarrhea, abdominal pain, fever	Intestinal tract and feces
Coxiella burnetii	Q fever	Chills, fever, weakness, headache, possible endocarditis	Infected cattle, sheep, and goats
Escherichia coli O157:H7	Gastroenteritis Hemolytic uremic syndrome (HUS)	Diarrhea, abdominal pain, bloody diarrhea Kidney failure, possible death	Intestinal tract and feces
Mycobacterium tuberculosis	Tuberculosis	Lung disease	Infected animals

Microbiology Analyses

Standard Plate Count (SPC):

- Aerobic plate count of total colony forming units of bacteria per milliliter or gram of raw, processed liquid and dry dairy products utilizing “SMEDP” 17th edition.

Bactoscan Analysis:

- an NCIMS approved alternate test for SPC (Standard Plate Count) on Raw Milk. Bacterial cells in a sample are lysed, liberated DNA is stained, and passed through light. The signal from the DNA is converted to an estimate of Bacteria and CFU’s in the original

Microbiology Analyses

Coliform Colony Forming Unit (CFU) Test:

- Agar plate method employing a media (VRB) optimizing the growth of coliform organisms

Salmonella:

- Test for rapid recovery of *Salmonella* in food, allowing detection and presumptive identification of the salmonella organism

Yeast and Mold Plate Analysis:

- Determination of yeast and mold cell forming colonies in a dairy sample under specified conditions utilizing “SMEDP” 17th edition.

Listeria:

- Test for detection and presumptive identification of the listeria organism

Microbiology Analyses

Direct Micro Count (DMC):

- Direct microscopic count examination of stained preparation of milk, or certain other dairy products, to identify and enumerate the number of bacterial clumps present

Lab Pasteurized Count (LPC):

- Raw milk in test tubes heated to 62.8C +/- 0.5C, survivors counted by standard plate count method utilizing “SMEDP” 17th edition.

Microbiology Analyses

Pre-Inc Plate Loop Count (PI):

- Raw milk sample undergoes preliminary incubation at 13 degrees Celsius for 16-18 hours before analysis to stimulate the growth of psychrotrophic organisms and subsequently a Plate Loop Count (PLC). Is performed utilizing “SMEDP” 17th edition.

Antibiotic Sensitivity Test:

- Test for sensitivity/resistance to antibiotics used in treatment of mastitis infections using the Kirby Bauer (agar diffusion) method.

Antibiotic Testing:

- Delvo, Charm II, Charm SL, HPLC

Microbiology Analyses

E-Coli:

- VRB-MUG Agar plate test

E-Coli 0157:H7:

- (AOAC Official Method) for identification and presumptive identification of E Coli H157:H7

Enterobacteriacea:

- Rapid differentiation of Enterobacteriaceae (eg. Klebsiella, E Coli, Salmonella, Shigella)

CP Staph

- Baird-Parker agar-BAM approved for food

PRESERVATION of MILK

METHODS

Pasteurization

Dehydration

Sterilization

1) **Pasteurization**

is a process of heating a food, which is usually a liquid(milk), to a specific temperature for a predefined length of time and then immediately cooling it after it is removed from the heat. This process slows spoilage caused by microbial growth in the food.

- Inactivation of bacterial pathogens (target organisms *Coxiella burnettii*)

- Assurance of longer shelf life (inactivation of most spoilage organisms and of many enzymes)

– Does not kill all vegetative bacterial cells or spores (*Bacillus* spp. and *Clostridium* spp.)

– Pasteurization temperature is continuously recorded

HISTORY OF PASTEURIZATION

- The French scientist Louis Pasteur invented pasteurization.
- To remedy the frequent acidity of the local wines he found out experimentally that it is sufficient to heat a young wine to only about 50–60 °C (122–140 °F) for a brief time to kill the microbes.
- Pasteurization was originally used as a way of preventing wine and beer from souring, and it would be many years before milk was pasteurized.
- Pasteurization of milk was suggested by Franz von Soxhlet in 1886.

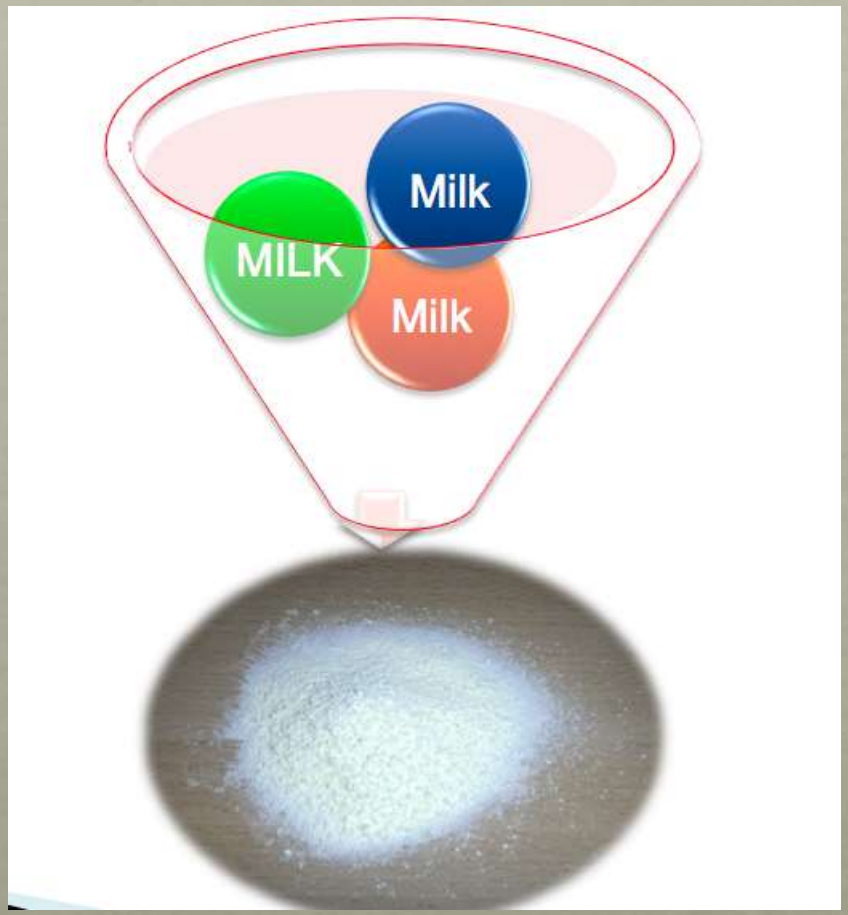
Louis Pasteur

Sterilization

is a term referring to any process that eliminates or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media.

Dehydration

Dehydrated milk is manufactured dairy product made by evaporating milk to dryness.



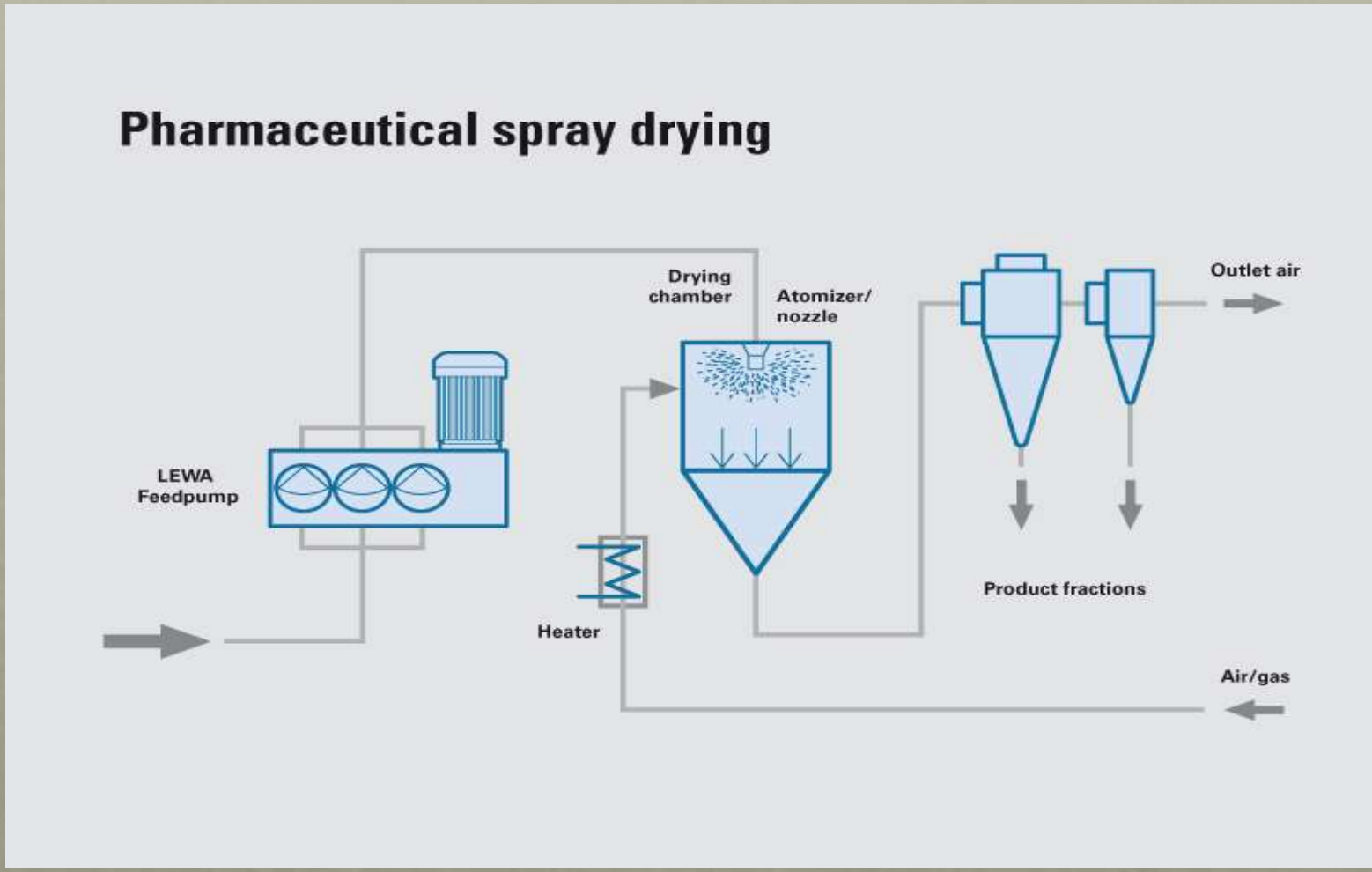
Dehydration

- Purpose: to preserve it; milk powder has a far longer shelf life than liquid milk and does not need to be refrigerated, due to its low moisture content.
- - First invented by Russian physician Osip Krichevsky in 1802.
- - Commercially available in 1832 by Russian chemist M. Dirchoff.

Milk drying methods

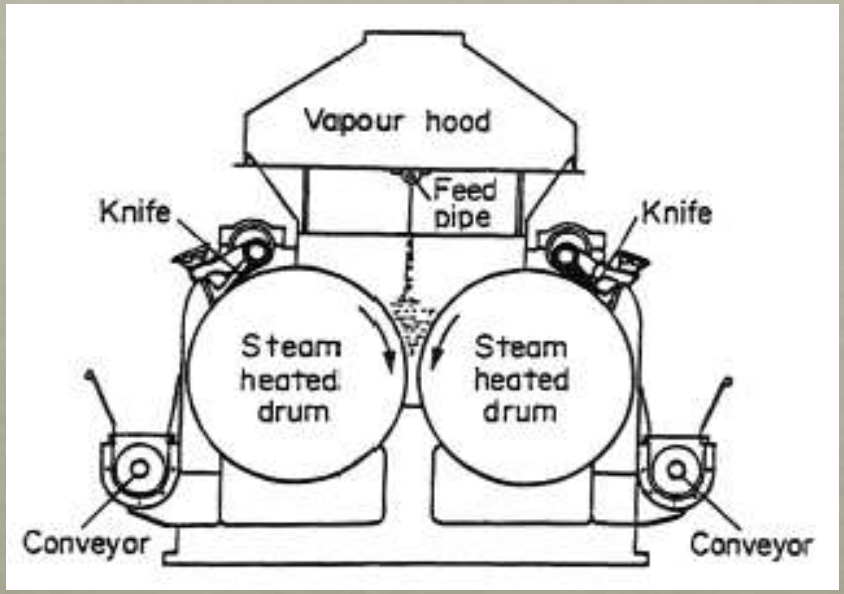
Spray Drying – Pasteurized milk is first concentrated in an evaporator to approximately 50% milk solids. The resulting concentrated milk is then sprayed into a heated chamber where the water almost instantly evaporates, leaving fine particles of powdered milk solids.

Spray Drying



Milk drying methods

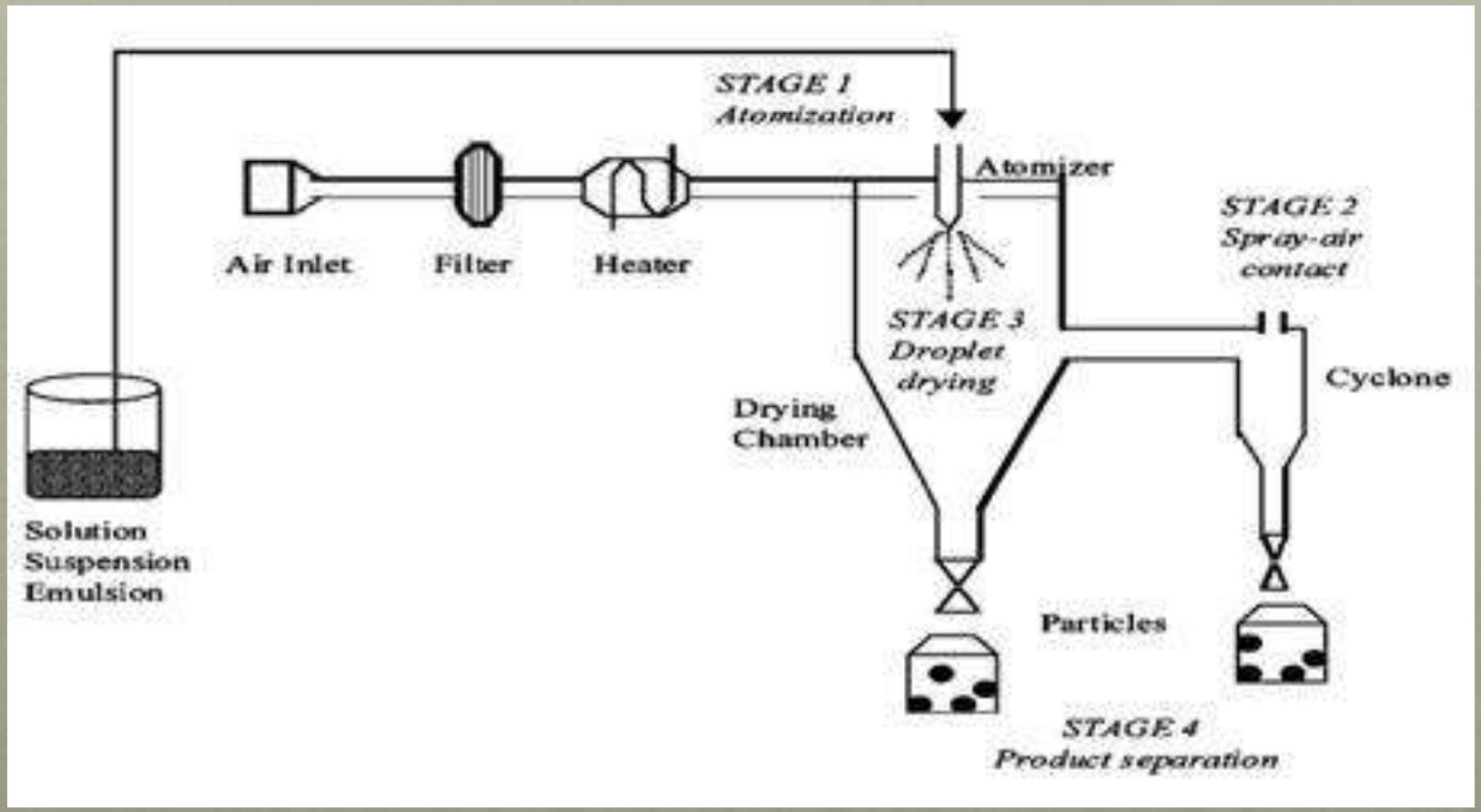
Drum Drying – Milk is applied as a thin film to the surface of a heated drum, and the dried milk solids are then scraped off. However, powdered milk made this way tends to have a cooked flavor, due to caramelization caused by greater heat exposure.



Milk drying methods

Freeze Drying – Same as drum drying but involves freezing which retains more amount of nutrition.

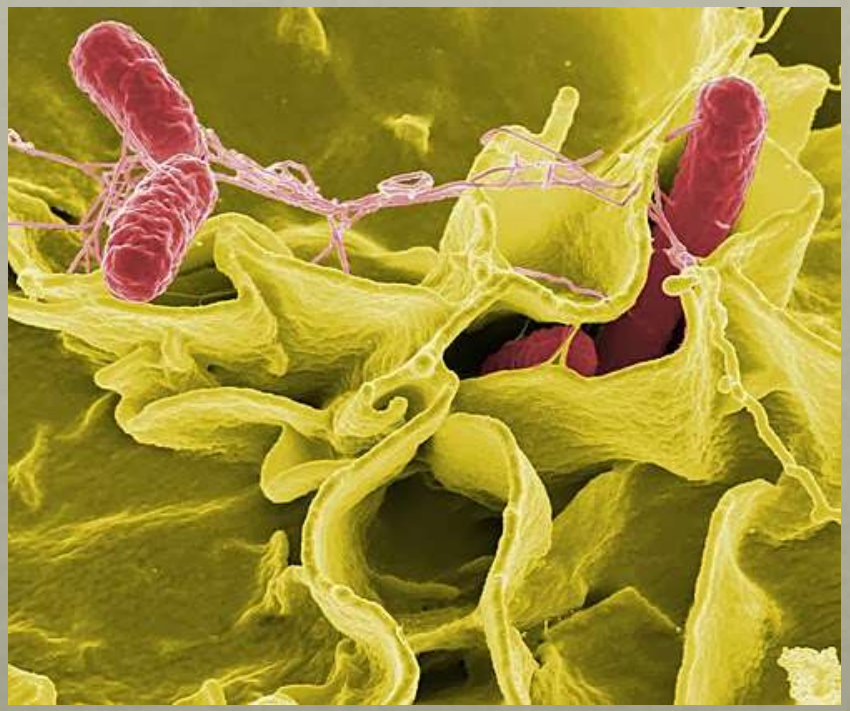
Freeze Drying



New bacteria discovered in raw milk

Chryseobacterium oranimense, which can grow at cold temperatures(7°C) and secretes enzymes that have the potential to spoil milk.“

C. haifense and C. bovis



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