COURSE TITLE: MICROBIOLOGY OF MILK PRODUCT

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MICROBIOLOGY OF THE INDIGENOUS MILK PRODUCTS PART-III

SOME LOCALLY IMPORTANT MILK PRODUCTS

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INDIGENOUS MILK PRODUCTS

- The term ‘indigenous milk products’ refers exclusively to dairy products of a particular region or country
- 47% of total milk products in India is converted to various indigenous products
- These products are the backbone of the Indian confectionary

Rabri is a sweet, condensed-milk-based dish, originating from the Indian subcontinent, made by boiling milk on low heat for a long time until it becomes dense and changes its colour to off-white or pale yellow. Jaggery, spices, and nuts are added to it to give it flavor. It is chilled and served as dessert.

Kheer is a type of pudding from the Indian subcontinent, made by boiling milk and sugar with one of the following: rice, broken wheat, tapioca, vermicelli, or sweet corn. It is flavoured with cardamom, raisins, saffron, cashews, pistachios, almonds or other dry fruits and nuts.

Dahi is a traditional yogurt or fermented milk product, originating from the Indian subcontinent, usually prepared from cow's milk, and sometimes buffalo milk, or goat milk. It is popular throughout the Indian subcontinent.

Shrikhand is an Indian sweet dish made of strained dahi.

Lassi is a popular traditional dahi-based drink that originated in the Indian subcontinent. Lassi is a blend of yoghurt, water, spices and sometimes fruit. Sweet and mango lassis are just like milkshakes. Bhang lassi is infused with the drug cannabis in the form of bhang.

Kulfi or Qulfi is a frozen dairy dessert originating in the Indian subcontinent in the 16th century. It is often described as "traditional Indian ice cream".

Ghee (Sanskrit: **Ghṛta**) is a class of clarified butter that originated in South Asia. It is commonly used in cuisine of the Indian subcontinent, Middle Eastern cuisine, Southeat Asian cuisine, traditional medicine, and religious rituals.

RABRI

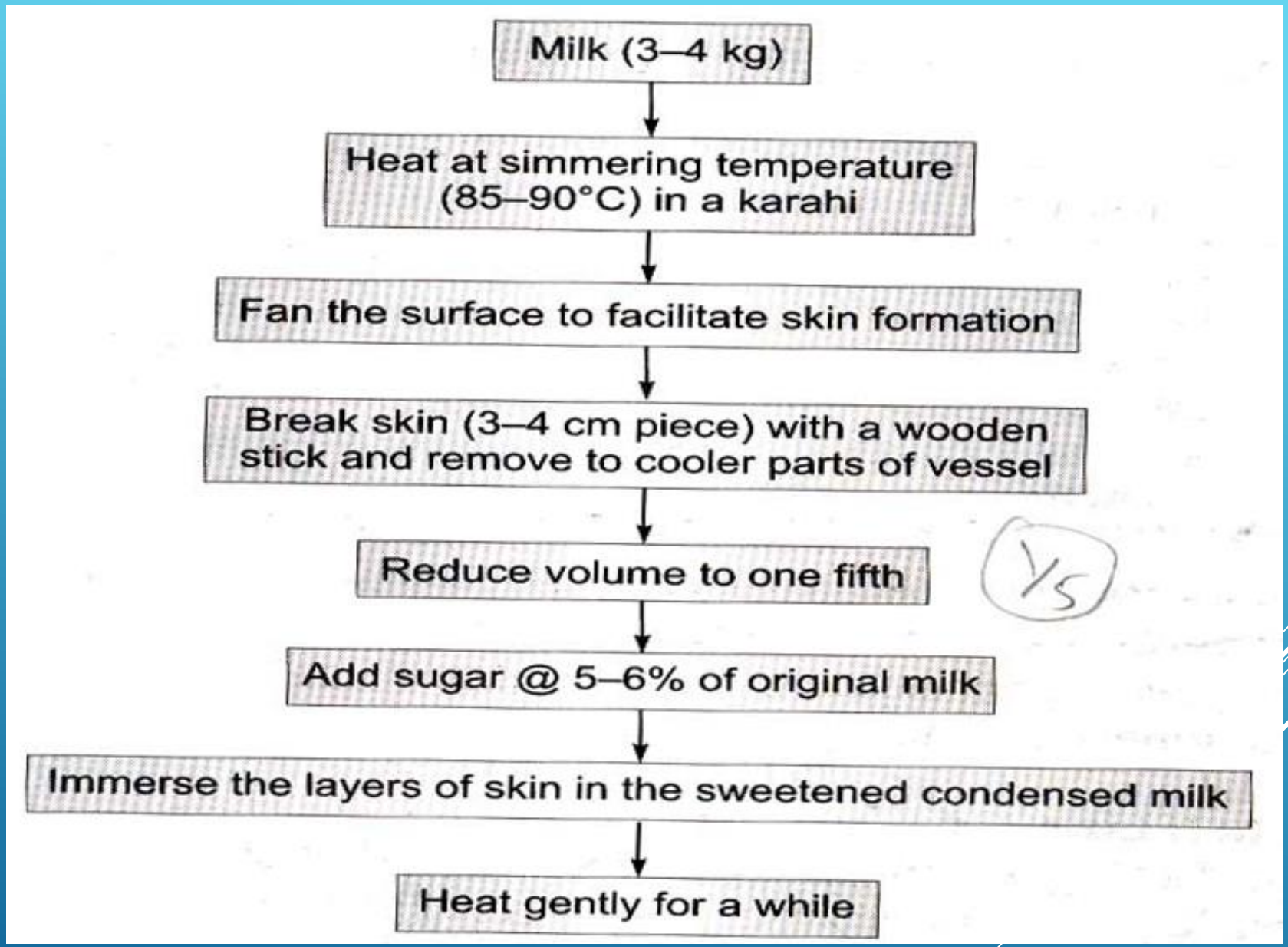
- A specially prepared concentrated and sweetened whole milk product containing several layers of clotted cream
- Produced in Northern and eastern regions of India
- Buffalo milk is normally used since it produces a more creamy and chewy consistency
- Fat and casein contents of buffalo milk contribute to the formation of a greater volume of creamy layer

Composition:

- Moisture 30%
- Fat 20%
- Protein 10%

Microbiological quality of rabri

- Singh et al (1975) examined 70 samples each of rabri and found higher mold count because of the higher moisture content
- *Penicillum*, *Aspergillus* and *Rhizopus* were the other commonly types of the molds in these products. *Alternaria* and *Cladosporium* appeared to occur only occasionally
- Mucor appeared to be most predominant type
- No food poisoning outbreak has so far been reported due to the consumption of these products



Manufacturing Process of Rabri

DAHI

- It is a product made in India and neighboring countries and resembles Yoghurt.
- Live cultures are deliberately used to produce therapeutic and health promoting properties in addition to nutritional benefits
- It inhibits the gastrointestinal tract infection, increase appetite, create anticarcinogenic effect in human system, lowers cholesterol level and help in digestion of lactose intolerance people.
- A part of lactose is converted into the lactic acid by the action of the starter culture, the presence of the lactic acid has a preservative effect on milk
- It has low pH value that inhibits the growth of the putrefactive bacteria and other harmful organism, thereby prolonging the shelf-life of the product

- During fermentation, partial splitting of proteins and greater availability of calcium, present in milk, enhances the nutritional value of the dahi
- It has a milk pleasant flavour and a clean acid taste
- It has a yellowish creamy-white color when made from cow milk and a creamy-white color when made from buffalo milk. It has a smooth and glossy surface
- The body is firm but not hard and free from gas holes

Composition:

- Fat 3.7%
- Caseins 2.8%
- Whey proteins 0.6%
- Lactose 4.8%

Different flavours:

- *Lactococcus Lactis, lactococcus diacetylactis, lactococcus cremoris* cultures along with the *leuconostoc* species used in most of the cases as a combination of the acid-producing and flavour-producing bacteria.

- They help in the production of a firm body and a sweet, mildly acidic flavour in the dahi which are highly desirable by consumers. *Leuconostoc* produces diacetyl that imparts buttery flavors to dahi.

Sometimes *Streptococcus thermophilous* and *lactobacillus acidophilus are used as* secondary culture in Dahi manufacture which imparts health-improving attributes/ probiotics properties to dahi.

Stages in manufacture of the dahi:

- Milk procurement: Hygienic production of the milk from healthy cows is prime requirement. For good microbiological control, collected pooled milk should be immediately chilled to 10˚C in 1 hour and < 5˚C in 2 hours inside bulk refrigerated milk tanks. Avoid unnecessary agitation to prevent lipolytic deterioration of milk flavour.

- Milk reception and storage in manufacturing plant: temperature of the raw milk at reception dock should be below4°C after quality checking of the raw milk.

- Separation of fat from raw milk is done and cream and skim milk may be added to standardized to desired fat level.

- Milk preparation: various ingredients to secure desired formulation are blended together at 10-50°C in a mix tank equipped with powder funnel and an agitation system in reconstitution vat.

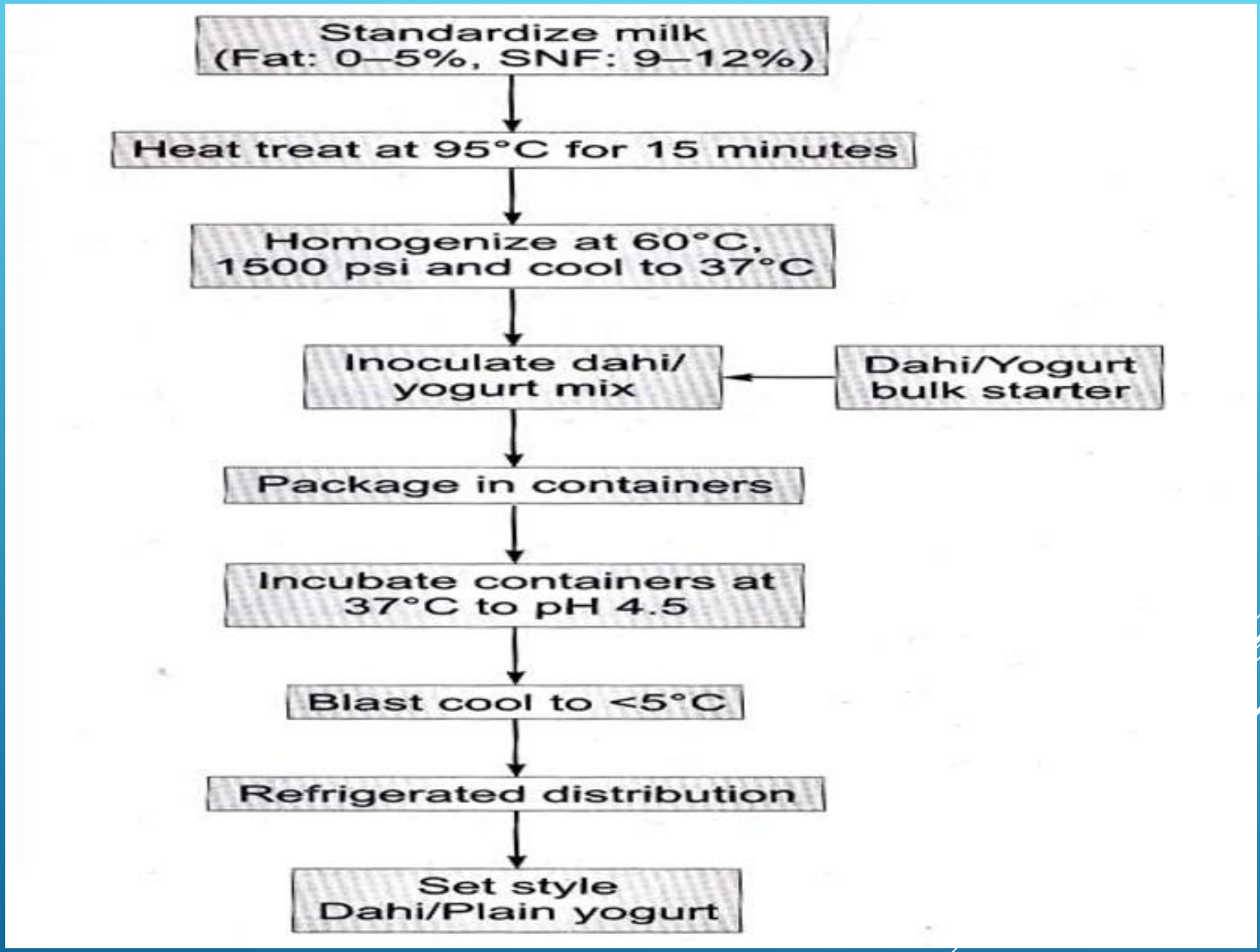
- Heat treatment: using heat exchanger, milk is heated to temperature of 85-95°C for 10-40 mins. Heating of the milk kills contamination and competitive microbes, produces growth factors by breakdown of milk proteins, generates microaerophilic conditions for growth of lactic organisms, and creates desirable body and texture in the cultured dairy products

- Homogenization: mix is passed through extremely small orifice at pressure of 2000-2500 psi, increases microbial count but not content and break down the fat globules

- Inoculation and incubation: the homogenized mix is cooled to an optimum growth temperature. Inoculation is generally at the rate of 0.5-5%. The inoculated milk is dispensed to cups and lids are applied. The cups are placed in trays and transferred to incubation room. The optimum temperature is maintained throughout incubation period to achieve a desired titratable acidity

- Cooling: the coagulated product is cooled down to 5°C, depending upon the product

- Storage and distribution: storage at 5°C ensures desirable shelf life by slowing down physical, chemical, and microbiological degradation



Flow diagram of Dahi manufacturing

- **The Bureau of Indian Standards has laid down the following specifications for dahi:-**

	Sweet dahi	Sour dahi
Acidity	0.7	1.0
Y/M count per g(max)	100	100
Coliform count per g (max)	10	10
Phosphatase test	-ve	-ve

- Sweet dahi*: Streptococcus lactis*, *S cremoris*, *S diacetylactis* (single or in combination)

- Sour dahi: Same culture as for sweet dahi, added with *lactobacillus bulgaris* or *S thermophilous* or both

Association of these microorganism may be attributed to the following effects:

- The ability to produce small amounts of hydrogen peroxide which inhibits or restrict the growth of other contaminating organisms.
- Less fastidious nutritional requirements.
- Their ability to grow in the presence of higher O_2 content
- Their compatibility and stimulatory action results in faster growth by utilizing limiting nutrients present in the milk.

Defects in dahi:Reason

- Green flavour: acetyldehyde accumulation by *lactococcus diacetylactis.*
- Yeasty flavour: yeast growth
- Rancid flavour: lipolytic activity

Contamination of dahi:

- yeasts are the most common contaminants in dahi than molds
- organisms get entry into the product from the atmosphere, utensils and human hands
- Incorporation of CO_2 (carbonation) into inoculated milk prolongs the shelf-life of dahi

- Coliforms, spore formers, pathogens and toxin producers

- In some reports, presence of *E coli*, *Staph aureus*, *Salmonela Paratyphi*, *Shigella dysenteriae was observed.* Survival periods of *Sal. Paratyphi* and *Sh.dysenteriae* were much shorter as compared to those of *E coli* and *Staph aureus* in the examined samples of Dahi.

- lactic acid and some other metabolites produced by fermentation process protect fermented milk from most gram-negative psychotrophic organisms.

KULFI

- **It is a popular frozen dessert (indigenous ice cream)**
- **It differs from ice cream in that it contains no air, so develop minor overrun**
- **The Bureau of Indian Standards has laid down the following specifications for kulfi**

Milk fat	**8.0**
Proteins	**3.5**
Acidity	**0.3**
Total colony count (per g) maximum	**250,000.0**
Coliform count (per g) maximum	**100.0**
Phosphatase test	**-ve**

- As per one study an average staphylococcal counts of $139X10^2$ per gram of kulfi were recorded in market sample
- Samples also showed presence of coliforms, staphylococci, psychrotrophs, enterococci and yeasts and molds in kulfi.
- Further investigations showed an average count of $95X10^3$ per gram of *Staphylococci* in kulfi in kulfi samples collected from push cart vendors and restaurants for local market of India.
- A number of isolates showed positive result for T DNase (thermostable), coagulase and enterotoxins A and B and the incidences were more in samples from push-cart vendors as compared to those restaurants
- A number of reports showed heavy contamination of market kulfi with staphylococci and other pathogenic bacteria.

- **Sources of the contamination:**
  - Additives like sugar, spices, fruits and flavour constitute one of the major source of the contamination
  - Addition of nuts such as almonds contaminated with *Aspergillus spp.* can cause aflatoxicosis.
  - Aerial contamination
  - Dust, water, flies and insects
  - Filling in improperly cleaned containers or cones
  - Use of bad quality of ‘sherbat’ which are added to the product before consumption
- **Shelf-life:**

Storage and distribution at a temperatureof -20 °C should maintain the sensory quality of the kulfi

GHEE

- It is a very important traditional milk product in Africa, the Middle East and India and neighboring countries including those of the Himalayan area
- Its main advantage over butter from which it is traditionally prepared is its superior keeping quality derived from the almost complete removal of water during the making process
- Boiling process destroys spoilage bacteria, all pathogens and inactivates some of the enzymes resulting from bacterial growth in the milk and butter

METHODS FOR THE PREPARATION OF GHEE

(i) From creamery butter

(ii) Directly from cream

- Unsalted creamery butter (commonly known as white butter) is heated in a ghee boiler
- Butter is first melted at low heat and then the steam pressure in the jacket is increased so that the mass begins to boil
- all the moisture has been removed the temperature of the liquid mass suddenly shoots up and the heating at this time has to be carefully controlled
- The end point is indicated by the appearance of a second effervescence, which is much finer than the first, together with the browning of the curd particles

- The final temperature of heating generally ranges from 110 to 120°C depending upon the practices in different regions

- After cooling and sedimentation the ghee is filtered through a muslin cloth to remove the sediment known as ‘ghee residue’ which consists mostly of burnt co-precipitates

- The product acquires the characteristic granular texture on cooling and is generally packed in tin containers, glass bottles and plastic pouches

- Cream is heated in a ghee boiler
- The procedure of heating and moisture removal, final temperature of clarification, cooling and sediment removal and granulation
- The direct cream method yields a higher quantity of ghee residue and takes a longer time

Fats & fatty acids	Amounts per 100 g of ghee[13]
Total fat	99.5 g
Saturated fat	61.9 g
Monounsaturated fat	28.7 g
Polyunsaturated fat	3.7 g
Trans fats	4 g
Omega-3 fatty acids	1.447 g
Omega-6 fatty acids	2.247 g
Omega-9 fatty acids	25.026 g
Other non-fat nutrients	**Amounts per 100 g of ghee**
Carbohydrates	0
Minerals	0
Cholesterol	256 mg (85%DV)
Phytosterols	0
Vitamin A	3069 IU (61% DV)
Vitamin B, C, D	0
Vitamin E	2.8 mg (14% DV)
Vitamin K	8.6 µg (11% DV)

Composition

	Cow	Buffalo
Milk fat (%)	99–99.5	99–99.5
Moisture (%)	0.2–0.5	0.2–0.5

Flavour/aroma of ghee

- Flavour of ghee is mainly due to the presence of the carbonyls, free fatty acids, lactones, active-SH groups, esters etc. carbonyls and lactones

- Yadav and co-workers have standardized a method for enhancing flavour in ghee through ripening of cream with *streptococcus diacetylactis* under optimum conditions

MICROBIOLOGICAL QUALITY

- High fat and low moisture, ghee appears to be inert product for survival and multiplication of microbes

- Mukherjee et al, isolated two species of *Aspergillus*, namely *A niger, A fumigatus* and *Penicillium gluacum*

- Bhat and Sethna isolated microbes are micrococci e.g *M flavus, M varians, Actinomyces rutgersensis, Bacterium linens, Bacillus Circulans, Bacillus megaterium, Aspergillus* and *Penicillium*

DEFECTS OF GHEE

Acidity: Since most of the bacteria isolated from ghee were either chromogenic, casein-hydrolytic or lipolytic, their activity could lead to development of acidity in ghee

Rancidity: The activity of microbes is an important factor in producing rancidity of butter and ghee, which may either be hydrolytic or ketonic type

Hydrolytic rancidity: The breakdown of fat into free fatty-acids and glycerol by the action of the microbial lipase secreted by bacteria and molds in ghee can cause hydrolytic rancidity in ghee *Aspergillus* and *Penicillium*. increase in acidity favours development of rancidity

- Sweet cream butter has lesser tendency to become rancid than sour cream butter
- The fat splitting enzyme, lipoprotein lipase, is found in milk
- it is sensitive to heat and is destroyed by proper pasteurization
- Lipase activity can deteriorate milk flavour rapidly. Homogenized, unpasteurized milk may become rancid in matter of minutes
- Even a small contamination of pasteurized, homogenizing milk with raw milk gives a rancid flavour
- Homogenizing the raw milk at 40 C appears to be greatest lipase activity
- Milk of old cows and milk near the end lactation appears to be higher in lipase activity
- Acceralation and foaming of raw milk accelerate hydrolytic activity

Ketonic rancidity: lower fatty acids such as butyric acid get converted into ketones, which give off-flavors. Mukherjee, explained the production of acetone from butyric acid by *Aspergillus niger*

Oxidative racidity: involves factors such as degree of the unsaturation in fat, availability of oxygen, heat, light, moisture content, free fatty acids, oxidation catalyst and antioxidants. Heating milk and cream to 75-85°C develops a cooked flavour

Fishy taints: fishy flavors and odors produced due to the production of the amines.Production of the fishy flavour is related to the conditions favorable for hydrolysis and oxidation of the lecithin

Acidity and salt combination favors development of the fishiness caused by *Pseudomonas ichthyosmus*, yeast and *Geotrichum. candidum*

Packaging:

Ghee is susceptible to deterioration from exposure to light, air and metals. It is packed in the tin-coated containers, polythene pouches and plastic film laminates and keeps in cool and dry place for long shelf-life

Shelf-life:

Milk fat provides a barrier to loss of moisture and air. It inhibits retrogradation of the starch (which is associated with staling). It should be stored in cool place, away from foods with strong odours and kept at 3°C

MAKKHAN

- The product made from buffalo milk has a harder/firmer body and a more granular texture than that from cow milk

- Composition:

Moisture	18–20%
Milk fat	78–81%
Curd	1.0–1.5%
Lactic acid	not> 0.2%

Makkhan contains vitamins A, D, E and K (fat soluble vitamins) and small amount of the fatty acids, arachidonic and linoleic acid.

Preparation:

- Raw material: buffalo milk is preferred because of the presence of the high fat content
- Processing: milk produced under rural conditions is heavily contaminated with bacteria. Some of these are fat splitting (lipolytic) and produce deleterious effect on makkhan. Heating for 5 to 10 mins should be done to inhibit the growth of the pathogens and spoilage causing microbes
- Curdling: the processed milk is converted into curd before churning it into butter
- Churning: curd is churned to separate the fat and mattha (buttermilk) by wooden churns

Microbiological quality:

- Microbes can enter in makhhan from aerial contamination, equipments, wooden churns are yeasts (sporogenous and asporogenous), molds, aerobic spore formers produces proteolytic and lipolytic organisms.
- Bacterial flora in makhhan are streptococci, coliforms and lactobacilli are also reported
- Asporogenous yeasts: C*andida*, *Torulopsis*, R*hodotroula and Cryptococcus*
- Sporogenous yeasts: P*ichia*, *Saccharomyces and Hanseula* Among molds, *Geotrichum*, *Aspergillus*, *Penicillium*, *Mucour and Syncephalastrum* have been reported

Defects of the makkhan:

- Rancidity
- Color defects:
- Bacterial discoloration: the causative organisms are *Pseudomonas nigrificans*, psychrotrophic organisms cause black discoloration.
- Fungal discoloration:
- Mold-origin:

  black (*Cladosporium, Aspergillus, Mucor, Alternaria*)
  brown (*Aspergillus, Phoma*)

  Green and blue green (*Penicillium, Aspergillus)*

  Orange and yellow *(Geotrichum candidum)*

  Reddish pink *(Fusarium)*
- Yeast origin:

  Black *(Torula*),

  Pink *(Rhodotorula)*

In order to improve the quality of traditional milk products the following suggestions may be considered:-

- Training for hygienic milk production should be given to farmers at farm gate level
- Dairy Technical Support Services should be provided
- Marketing of traditional milk products should be channelized through identified institutions.
- Improved breeds of animal should be introduced or given to farmers

FUTURE FOR TRADITIONAL DAIRY PRODUCTS

- The high value that the consumers attach to these products is a guarantee that will ensure the future growth and modernization of traditional dairy products
- The many advances that dairy technology has made will provide the tools to further explore and improve upon the quality and shelf-life of these time-tested products
- The advent of convenience foods and their increased acceptability in the region will further support the modernization of this sector

THANK YOU