

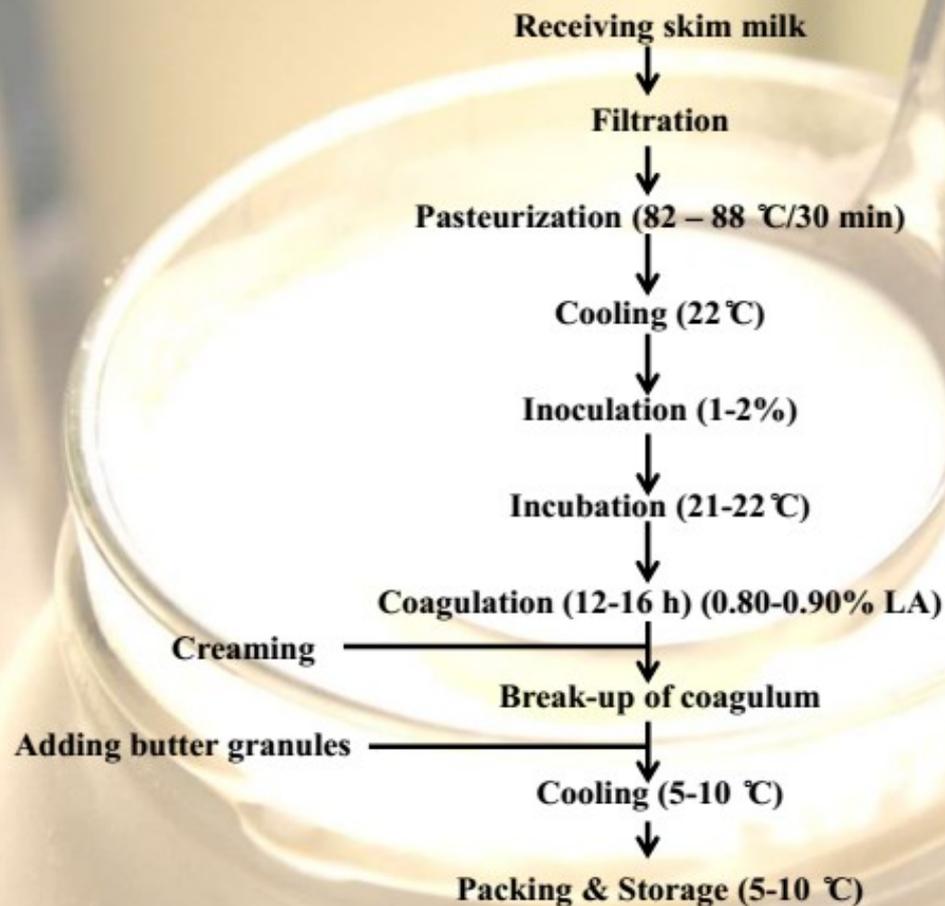


Cultured Buttermilk

- 1. Drinking of butter after churning dahi in to country butter is a very common habit in India.**
- 2. This product has most of the fermented milk solids except fat which goes in butter. It has mixed LAB, especially *Lactococci* and *Leuconostoc* which gives it typical diacetyl flavour.**
- 3. Manufacturing cultured butter milk on industrial scale involve selection of good quality raw material, standard cultures and optimized process of fermentation, packaging and storage.**
- 4. True buttermilk is the fluid remaining after cream is churned into butter.**
- 5. Cultured butter milk is prepared by souring true butter milk or more commonly, skim milk with a butter starter culture that produces a desirable flavor and aroma.**
- 6. Cultured butter milk is prepared with the help of normal mesophilic lactic acid bacteria.**

<u><i>Lactococcus lactis</i> subsp. <i>lactis</i></u>	D-type
<u><i>Lactococcus lactis</i> subsp. <i>cremoris</i></u>	
<u><i>Lactococcus lactis</i> subsp. <i>diacetylactis</i></u>	
<u><i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i></u>	L-type
<u><i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i></u>	

MANUFACTURE OF CULTURED BUTTERMILK



Making of Cultured buttermilk

- 1. Selection of milk:** The quality of raw material decides the quality of final product. The raw milk selected for CBM manufacture should have normal composition, be free from off flavor and odours and free from inhibitory substances. It should have lower microbial count.
- 2. Standardization of milk:** Standardize milk for fat and solids -not-fat content looking to legal requirements and also as per consumer demands. Skim milk is the starting material and it may be added with approximately 1.7% fat. In certain commercial processes, fat is added as granules in cold fermented butter milk. Sodium chloride at the rate of 0.1-0.2% and sodium citrate at the rate of 0.1-0.2% may be added for enhancement off-flavor.

- 3. Heat treatment:** Proper heat treatment of at least pasteurization equivalence is mandatory for CBM production. This is to ensure destruction of pathogens and make the product safe for human consumption. It also helps in providing suitable environment for the cultures. Heating at 85 C for 30 min is the optimum heat treatment for CBM production which gives best quality product in terms of flavour and shelf-life. Heating below 82 C or above 88 C causes a weak body, that allows whey separation in cultured butter milk.
- 4. Cooling:** Milk must be cooled down to inoculation temperature as soon as holding period is over. Inoculation in hot milk will destroy the culture. It should be 22-25 C for cultured buttermilk.

- 5. Inoculation:** The milk should be inoculated with appropriate mesophilic starter culture just after cooling. Inoculation rate varies from 0.5-2.0%, depending upon the starter activity and time of incubation. At the time of inoculation, starter should have 0.80 -0.85% acidity. Over ripe culture are not suitable as the lactic streptococci have passed their peak of acidity. If it is under ripened, <0.8% lactic acid, it will not have sufficient number of aroma bacteria.
- 6. Incubation:** The typical incubation temperature for CBM is 21.6 C. There are several reasons for selecting 21-22 C as incubation temperature.
- Any departure of more than a degree or two above or below will cause imbalance in the bacterial mixture and will adversely affect the product.
 - At lower temperature, the starter organisms grow too slowly, and curdling is delayed excessively.
 - At much higher temperature, the streptococci overgrow aroma bacteria and product lack flavor and aroma.

- ❑ At higher incubation temperature the curd forms at a lower acidity and it tends to shrink, causing whey separation.
- ❑ Use of lowest practicable temperature inhibits the growth of undesirable bacteria that survive the heat treatment given to the milk.

7. Cooling, Agitation and Dilution: After desired stage of ripening, the curd must be cooled rapidly to avoid over ripening. Mixing may be necessary to hasten cooling, but it should not be done in a manner that incorporate air. Excessive agitation decreases the stability of butter milk and increases whey separation. Gentle agitation is required to break curd and to have efficient and quick cooling. Here, dilution of the product can also be done to adjust solids level to desired level. Some manufacturers also add some spices and condiments to flavour it. The final cooling of the product done at less than 5C.

8. Packaging and Storage: Bottle/cartons are commonly used. Packaging material shouldn't increase the microbial load in the product. Product will have better shelf-life if stored below 5C.

Composition of CBM:

Item	Concentration
Total solid	10%
Fat	0.88%
Protein	3.3%
Ash	0.9%
Lactose	4.80%
<u>Titratable acidity</u>	0.80%
pH	4.5

Characteristics of CBM:

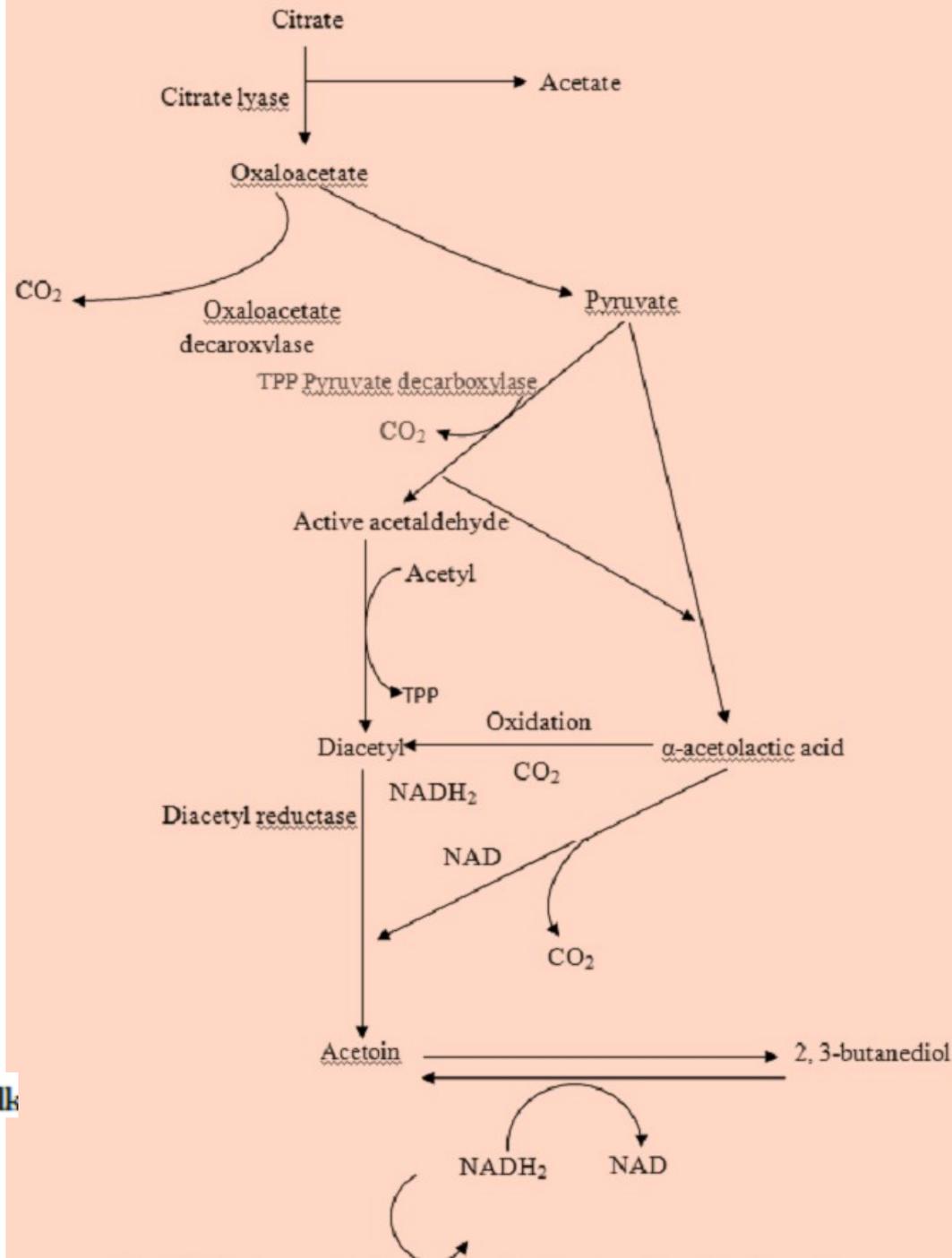
- ❑ A good quality butter milk, after packaging has a pH 4.5 and possess smooth viscous body giving a slow even flow when poured.
- ❑ The flavor should be clean acid with an integrated aromatic diacetyl and free volatile acid background.
- ❑ It exhibits no free whey/whey separation. The keeping quality of good buttermilk at 5 C is approximately 2 weeks
- ❑ In India, standards for contamination prescribed: Coliform count should be <10 per ml and yeast & mold count should be <100/ml.

- ❑ The product has a fine delicate flavour of diacetyl with acidic taste.
- ❑ The principal flavouring component in cultured butter milk is diacetyl produced by the aroma bacteria. This is produced by fermentation of citric acid (about 0.2% present in fresh milk).
- ❑ To prevent deterioration of fine flavor, citric acid may be added (0.1-0.2%) to milk before pasteurization to provide sufficient substrate and maintain diacetyl levels between 2-4 ppm.
- ❑ A proper balance of different flavour compounds namely diacetyl, acetoin, acetate and CO₂ will give a characteristic flavor to the product.
- ❑ *L. lactis* subsp *diacetylactis* and *Leuconostoc* are the major diacetyl producing microorganisms in cultured butter milk.
- ❑ Neither *L. lactis* subsp. *diacetylactis* nor *Leuconostoc* can use citrate as an energy source. However, they produce different end products from citrate.

- ❑ *L. lactis* subsp *diacetylactis*, however grows better in milk than *Leuconostoc* and accumulates high concentration of diacetyl (up to 5 ppm). It can therefore be used to produce both acid and aroma. This organism unfortunately produces acetaldehyde, an undesirable flavor in butter milk (described as green or yoghurt like off flavour).
- ❑ On the other hand, *Leuconostoc* don't have this disadvantage and they help to scavenge acetaldehyde.

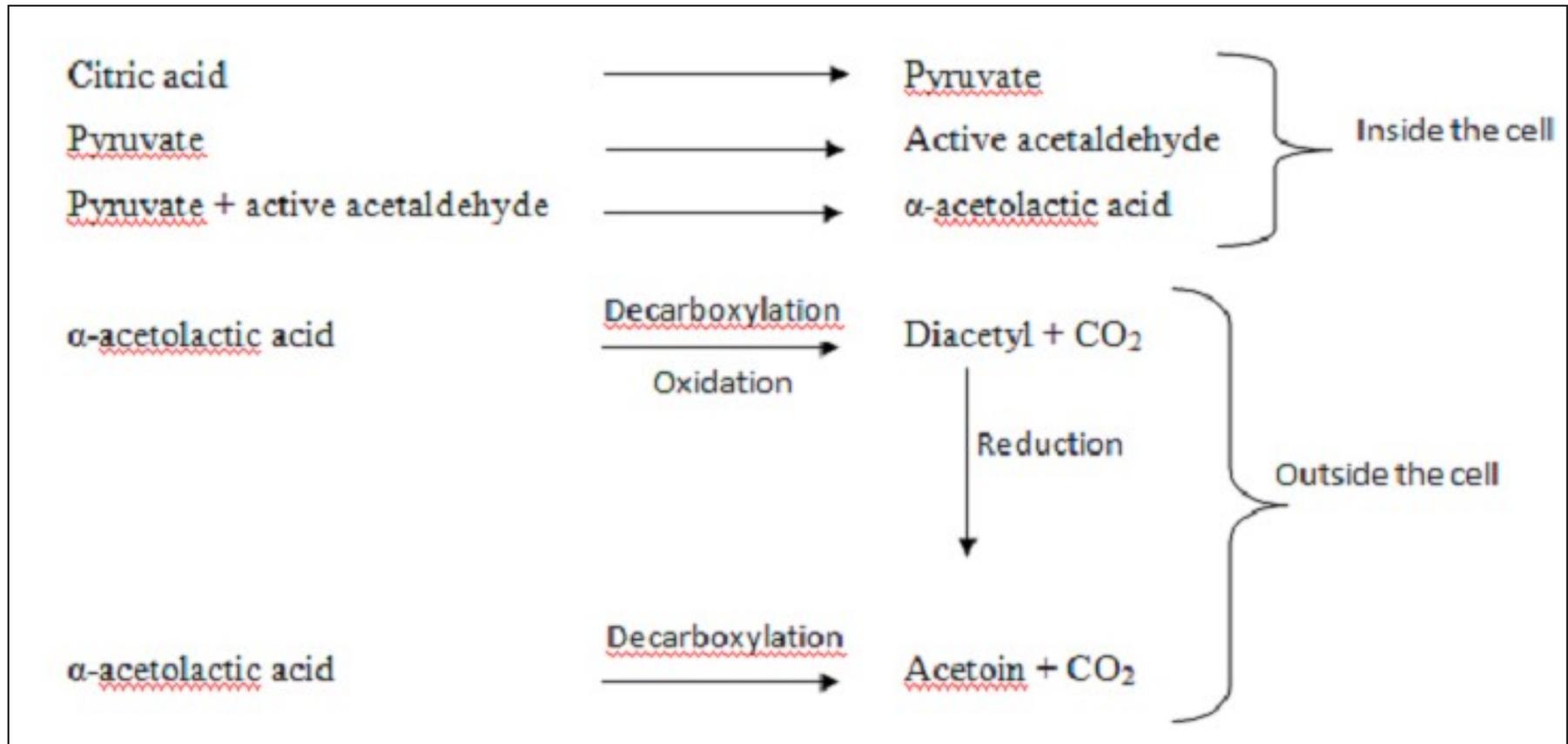
Modes of diacetyl production

- Diacetyl is produced by fermentation of citric acid by selected LAB in milk.
- First theory explains that diacetyl is formed by oxidative decarboxylation of α -acetylactate which is excreted in to milk by bacterial cells.

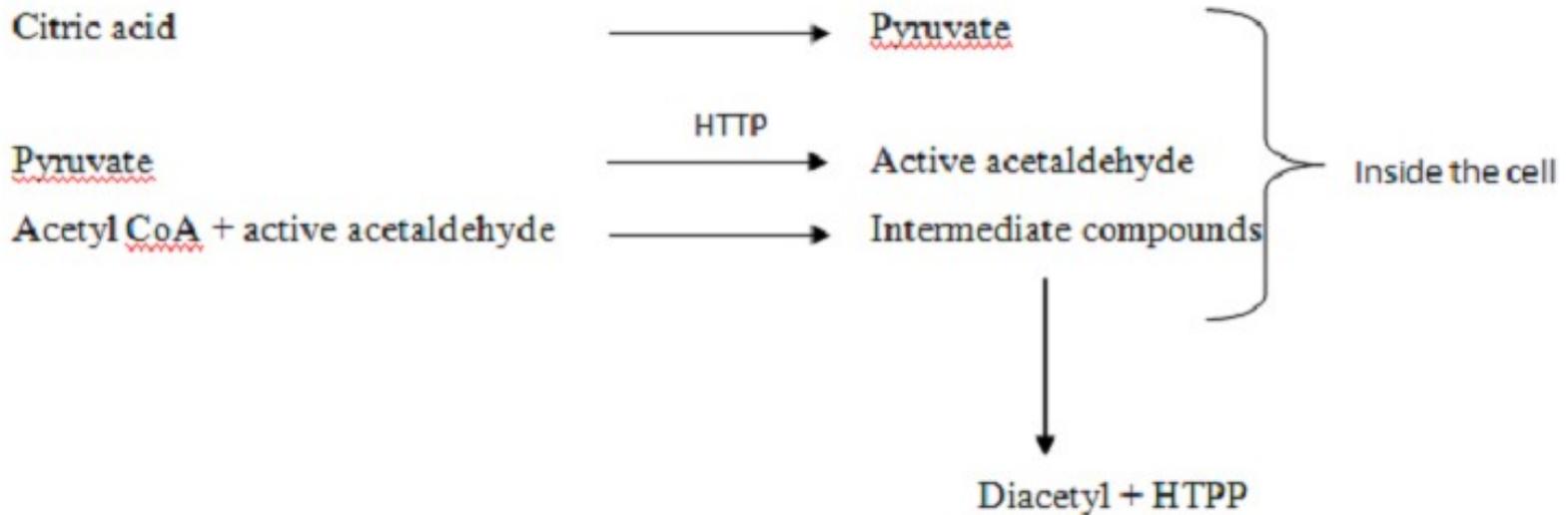


Pathways for flavour production in cultured butter milk

According to Van Beynum Pette (1938); Deman (1956); Seitz et al. (1963) the diacetyl is formed by oxidative decarboxylation of α -acetolactate which is excreted into milk by bacterial cells.



2nd theory, According to Speckman & Collins (1968) and Cogan (1984) diacetyl is formed in the bacterial cells by reaction of acetyl CoA and activated acetaldehyde. This process occurs totally intracellularly and not via α -acetolactate.



Factors affecting diacetyl content in cultured butter milk:

- ❑ **Type of culture and culture balance:** Cultures inherent physiological properties decides the metabolism. The balance among different strains and especially the proportion of acid and aroma producers in culture decides the fate of diacetyl.
- ❑ **Citric acid concentration in milk:** Citrate is the substrate as well end product inhibitor of the diacetyl. Surplus citrate in the product after fermentation helps stabilize diacetyl content.
- ❑ **Acidity and pH:** The metabolism of citrate involving various enzyme activities depends mainly on pH of the medium. Further, diacetyl in cultures like *Leuconostocs* is produced externally from α -acetolactate in presence of lactic acid. So, pH 4.6 is optimum for stabilizing the concentration of diacetyl.
- ❑ **Aeration and oxygen content in milk:** The diacetyl and acetoin can easily reduce to 2,3 butanediol (flavourless). Since these compounds interchange based on oxidation-reduction potential, aeration and oxygen are important.

- ❑ **Type of packaging material:** It decides permeability for oxygen. When bottles are used as packaging material, there can be decrease in diacetyl; while in polyethylene pouches, because of permeability to oxygen, the diacetyl may remain stable.
- ❑ **Concentration of manganese and magnesium in milk:** These ions act as co-factors for key enzyme activities in metabolic pathway hence; affect the concentration of end product.
- ❑ **Age of milk and other contaminants:** If milk is stored and other contaminants which can produce diacetyl reductase are present, it may reduce flavour.
- ❑ **Temperature of incubation:** The growth of cultures and the activities of enzymes in flavour production pathway depend on temperature.
- ❑ **Agitation and cooling:** It can affect the growth of culture as well as oxygen content in product and hence the diacetyl conc. Fast cooling and aeration for 1 h has favourable effect. Too slow cooling in incubation vessel has adverse effect. If not cooled immediately, especially in large tanks, diacetyl may quickly decrease.

Advances in cultured butter milk technology:

- ❑ Double stage homogenization of milk for 1.7% fat butter milk adds to richness of flavor.
- ❑ Controlled fermentation process involving the chilling of buttermilk at pH 5.2 to arrest the growth of added *L. diacetylactis* followed by acidification of the product to pH 4.5. Added lactic acid has been reported to give fine flavor through increased diacetyl, but without developing acetaldehyde.
- ❑ Various types of flavoured butter milks can be produced with incorporation of natural fruits or essences.
- ❑ To improve texture and viscosity of the product, membrane filtration technology can be used. Concentration of milk by UF increases protein and decrease lactose content.
- ❑ Vacuum de-aeration before cooling for removal of excess carbon dioxide, helps to get better flavor in CBM.

Defects in cultured butter milk:

Flavour defects:

- ❑ High incubation temperature (27°C) discourages growth of flavor producers; therefore insufficient diacetyl will be present. Such defects can be prevented by ensuring that the acid producing culture is active, because the flavor producers will be activated only after sufficient acid has been produced (0.8–0.85%, pH 5) and incubating at 22°C.
- ❑ Milk should be supplemented with citrate, and after the curd has been broken at the optimum pH, the product should be rapidly cooled with gentle agitation. This will prevent degradation of diacetyl.
- ❑ If incubation is not monitored and fermentation not halted by cooling, acid production will continue and may even exceed 1%. This process produces a highly acidic product with a loss of diacetyl. Excessive acidity will also lead to wheying off because of a lowered water-holding capacity of the proteins.

Body defects:

- ❑ If the culture lacks adequate activity and if the product is cooled at low acidity, the finished product will not have optimum viscosity. In contrast, excessive viscosity can result from cultures such as *L. lactis* subsp. *lactis*, which form long chains. Some contaminants produce slime, which results in a highly viscous product.