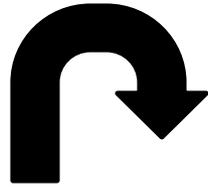




Accelerated Ripening of Cheese

Why needed?

Ripening is an expensive and time-consuming process



Extended ripening period involves increased cost due to refrigerated storage, space, labour, loss in weight and higher capital cost

Cheddar cheese is typically ripened for 6-9 months while Parmesan is usually ripened for two years.

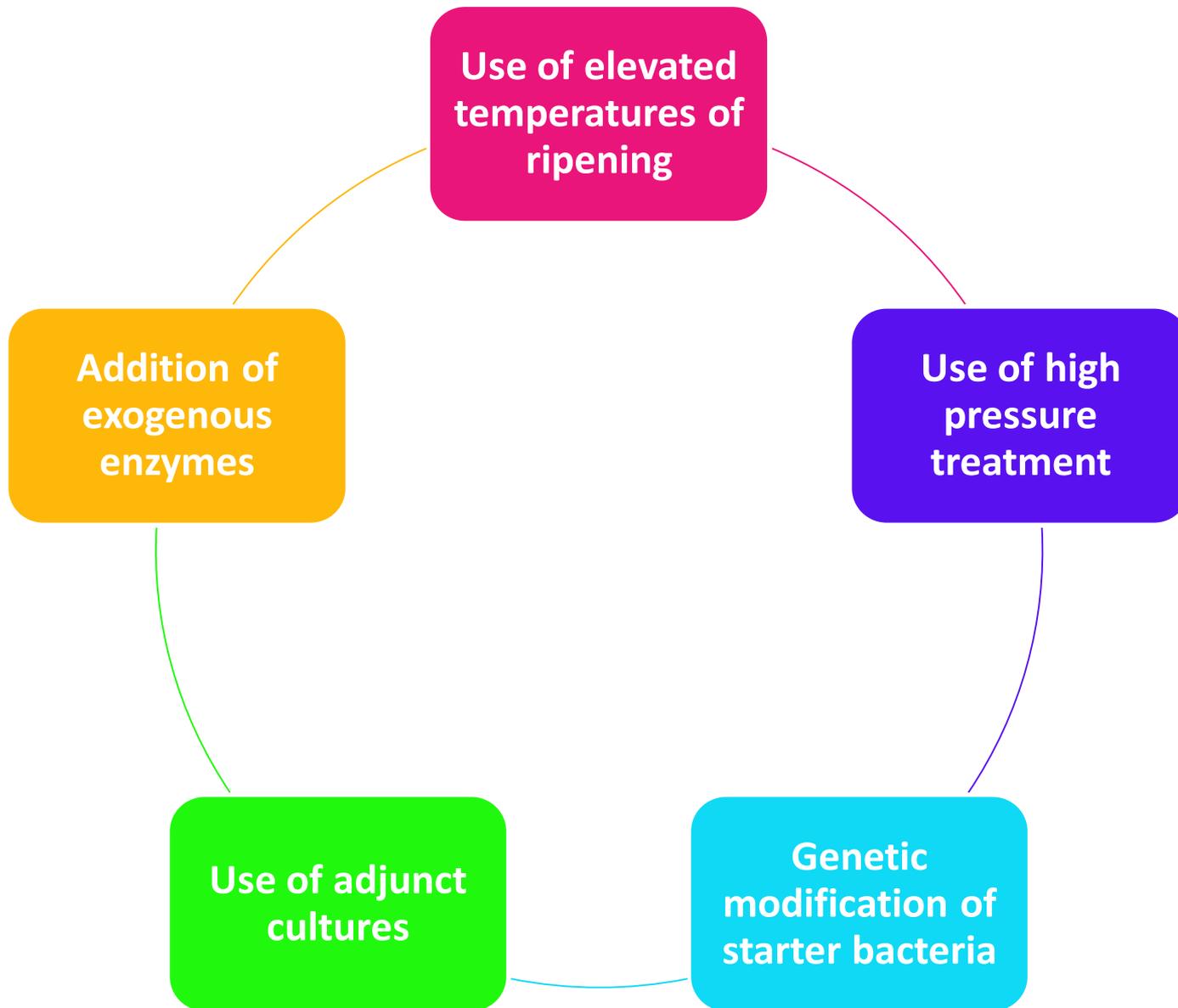


Acceleration of cheese ripening

- ❖ Biochemical changes which occur during ripening are slow and take about 6 months to years, depending on the variety of cheese.
- ❖ For example, Cheddar cheese is typically ripened for 6 -9 months while Parmesan is usually ripened for two years. Such extended period of ripening involves capital investment in form of product and utilities like storage rooms, refrigeration etc.
- ❖ Therefore, various technological interventions are applied to shorten ripening period. This process of reducing normal ripening period is called as **accelerated ripening.**

Methods of Accelerated Ripening:

- **Ripening is a slow and consequently, expensive process that is not fully controllable.**
- **Economic and possibly technological incentives to accelerate ripening are: an elevated ripening temperature, exogenous enzymes, modified starters and adjunct cultures.**
- **Accelerate ripening may be used to modify flavor and in effect to create new varieties.**
- **Slow flavor development and low flavor intensity are major problems with reduced-fat cheese and methods used to accelerate ripening are applicable to low fat cheese also.**



Use of elevated temperatures of ripening:

- **Cheese is ripened at uniform temperature and humidity, depending on variety of cheese. Ripening temperatures are as follows:**
 - ✓ **Emmental, 22-24°C**
 - ✓ **Mold and smear-ripened cheeses, 12-15°C**
 - ✓ **Dutch varieties, 12-14°C**
 - ✓ **Cheddar, 6-8°C**
- **Temperatures are maximum and usually maintained for 4-6 weeks to induce growth of desired secondary microflora.**
- **Ripening temperatures of most varieties in no case is more than 20-25 C as keeping cheese above this temperature causes texture to be too soft, deforms readily, cause exudation of fat and excessive loss of moisture.**

- **Accelerating ripening of most cheese varieties by increasing ripening temperature is quite limited except Cheddar cheese (ripened at 6-8°C).**
- **Limited to cheese which is made from good quality pasteurized milk and is microbiologically safe.**
- **Accelerating ripening is simplest and cheapest method as no additional costs are involved rather there may be savings due to reduced refrigeration costs.**
- **Cheddar cheese can be ripened at elevated temperatures. E.g, duration of ripening can be reduced by 50% by increasing ripening temperature from 6-13°C. Highest temperature that can be used is 16°C, 20°C could be used for a short period; 12-14°C is probably optimal.**
- **Ripening can be accelerated or delayed by raising or reducing temperature at any stage during process.**

Addition of Exogenous Enzymes:

- **Cheese ripening is an enzymatic process and ripening is accelerated by augmenting activity of key enzymes.**
- **Addition of single enzyme, which accelerates one particular reaction, is unlikely to produce a balanced flavor. So, a need for addition of mixture of enzymes in proper ratio is required.**
- **Addition of combinations of various fungal proteases and lipases to Cheddar cheese reduce ripening time by 50%. Lipase in combination with proteinase produced low levels of bitterness.**
- **Lipase/proteinase preparation derived from *Aspergillus oryzae* released C6-C10 fatty acids to produce typical Cheddar cheese flavor.**

a. Coagulant:

- **Proteinases in coagulant is responsible for primary proteolysis so, ripening could be accelerated by increasing the level or activity of rennet in cheese curd.**
- **Chymosin is limiting proteolytic agent in initial production of amino nitrogen in cheese, so increasing level of rennet in cheese curd does not accelerate ripening and causes bitterness.**
- **Chymosin produces only relatively large oligopeptides which lack typical cheese like flavor and may be bitter. Chymosin-produced peptides are hydrolysed by bacterial (starter and non-starter) proteinases and peptidases and hence, increased chymosin activity to be coupled with increased starter proteinase and peptidase activities in order to accelerate ripening.**

- Chymosin has very little activity on β -casein, probably because the principal chymosin susceptible bond in β -casein, Leu192-Tyr193, is in the hydrophobic C-terminal region of the molecule which appears to interact hydrophobically in cheese, rendering this bond inaccessible.
- *Cryphonectria parasitica* proteinase preferentially hydrolyses β -casein in cheese (possibly because its preferred cleavage sites are in the hydrophilic N-terminal region) without causing flavor defects.
- Rennet containing chymosin and *Cryphonectria parasitica* proteinase might be useful for accelerating ripening.

b. Plasmin:

- **Plasmin contributes to proteolysis in cheese, especially in high-cooked varieties in which chymosin is extensively or totally inactivated.**
- **Plasmin is associated with casein micelles in milk, which can bind at least 10 times the amount of plasmin normally present, and totally and uniformly incorporated into cheese curd, thus overcoming major problem with use of exogenous proteinases to accelerate cheese ripening.**
- **Addition of exogenous plasmin to cheese milk accelerate ripening of cheese without off-flavor development.**

c. Other proteinases:

- **Exogenous (non-rennet) proteinases has attracted considerable attention.**
- **Acid proteinases caused pronounced bitterness but addition of certain neutral proteinases and peptidases with salt gave marked increase in flavor after one month at 20°C but an overripe, burnt flavor and free fluid came after one month at 32°C.**
- **Incorporation of enzyme-treated cheese in processed cheese increase Cheddar flavor at 10% addition and a very sharp flavor at 20%.**
- **Good quality medium sharp Cheddar could be produced in 3 months at 10°C through addition of combination of selected proteinases and lipases.**

- **With the exception of rennet and plasmin (which adsorbs on casein micelles), incorporation and uniform distribution of exogenous proteinases throughout cheese matrix poses several problems:**
 - **Proteinases are usually water-soluble, and when added to cheese milk, most of the enzyme is lost in whey, which increases cost**
 - **Enzyme-contaminated whey must be heat-treated if whey proteins are recovered for use as functional proteins.**
 - **Amount of Neutrase added to milk ensure sufficient level of enzyme in curd reduces the rennet coagulation time, yields a soft curd in which at least 20% of β casein is hydrolyzed at pressing and reduces cheese yield.**

d. Exogenous lipases:

- **Lipolysis contributes to flavor development in strong-flavored cheeses (hard Italian, Blue varieties, Feta).**
- **Rennet paste or crude preparations of pre-gastric esterase (PGE) are normally used in production of Italian cheeses.**
- ***Rhizomucor miehei* lipase may be used for Italian cheeses, lipases from *Penicillium roqueforti* and *Penicillium candidum* may also be satisfactory.**
- **The ripening of blue cheese may be accelerated and quality improved by added lipases. A blue cheese substitute for use as an ingredient for salad dressings and cheese dips can be produced from fat-curd blends by treatment with fungal lipases and *P. roqueforti* spores.**

d. β -Galactosidase (Lactase):

- β -galactosidase (lactase) hydrolyses lactose to glucose and galactose, results in stimulation of lactococci and shortens lag period growth of lactococci.
- Lactose hydrolysed cow and buffalo cheese milks reduce manufacturing time, improve flavor and accelerate ripening.
- Lactase from *Kluyveromyces lactis* available as Maxilact contains a proteinase, which is responsible for increased levels of peptides, and free amino acids.

Use of Adjunct Cultures:

- **Adjunct cultures are specifically selected strains, which are intentionally added to accelerate ripening of full fat cheese and for flavor enhancement of low fat cheese.**
- **Decrease bitterness**
- **Addition of different *Lactobacillus* spp. (*L. casei* and *L. plantarum*) to Cheddar cheese milk to a level of 10^5 - 10^6 cfu/ml increase levels of free amino acids to attain highest flavor scores.**
- **Flavor development and biochemical changes in buffalo milk Cheddar cheese is faster when *L. casei* is supplemented with cheese.**
- **Principal adjuncts used in accelerated ripening of cheese are mesophilic lactobacilli and thermophilic lactobacilli.**

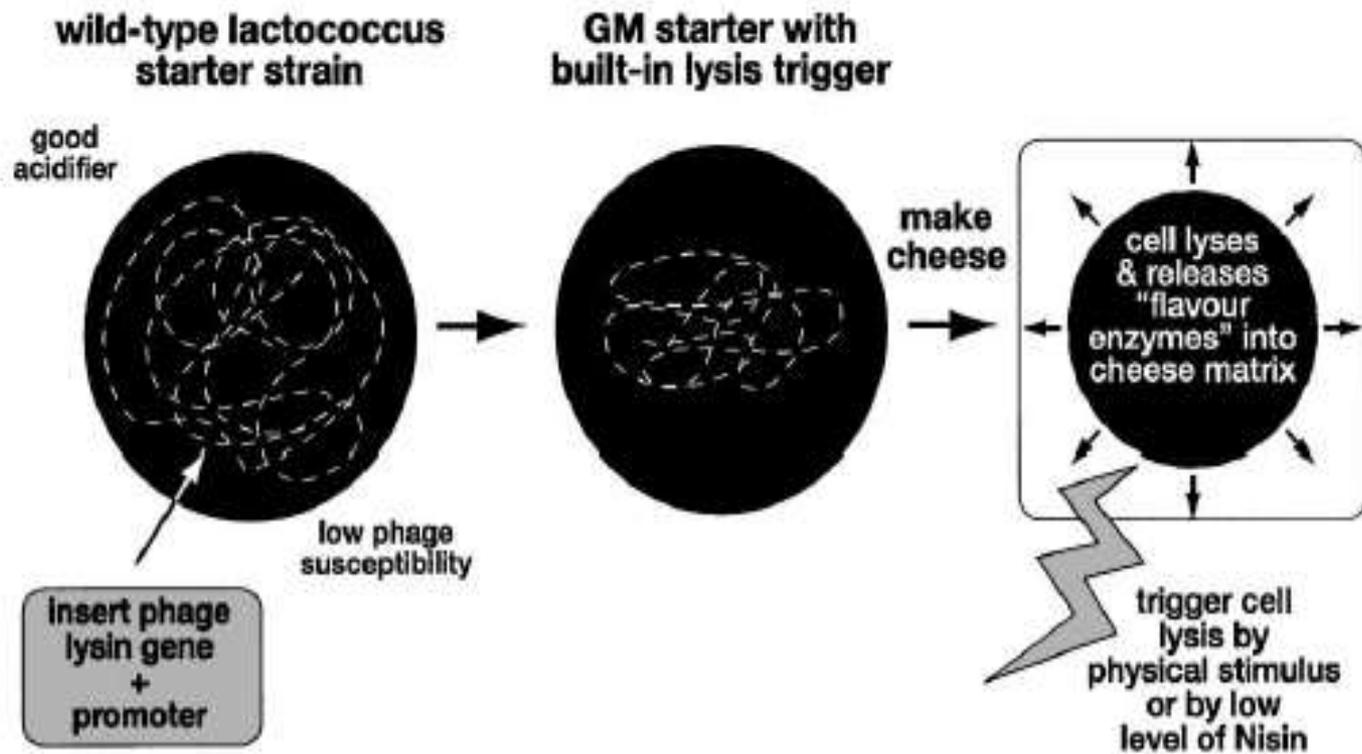
- **Inoculation with mesophilic *Lactobacillus* adjuncts enhanced flavor and accelerated proteolysis at level of small peptides and amino acids.**
- **Mesophilic lactobacilli modify proteolysis; they result in a higher concentration of free amino acids and improve the sensory quality. In contrast to mesophilic lactobacilli, thermophilic lactobacilli die rapidly in cheese, lyse, and release their intracellular enzymes.**
- **Cheeses made with thermophilic *Lactobacillus* spp. as starters contain high concentrations of amino acids. Although thermophilic lactobacilli will not grow in Cheddar cheese, their inclusion as starter adjunct intensifies flavor of Cheddar.**

Modification of starter bacteria/Starter cultures



Genetically engineered starters:

- **Food-grade controlled lysis of *Lactococcus lactis* for accelerated cheese ripening is an important approach. To accelerate cheese ripening is to induce lysis of *L. lactis* starter strains for facilitated release of intracellular enzymes involved in flavor formation.**
- **Controlled expression of lytic genes *lytA* and *lytH* encoding lysin and holin proteins of lactococcal bacteriophage phi-US₃ respectively, was accomplished by application of food -grade nisin-inducible expression system.**
- **Production of lysin and holin is essential to obtain efficient lysis and release of intracellular enzymes as exemplified by complete release of the debittering intracellular aminopeptidase N. Production of holin alone leads to partial lysis of host cells, whereas production of lysin alone does not cause significant lysis.**



GM organism designed to lyse in cheese “on demand”

Stimulation of starter cells

- ❑ Growth of starter cells may be stimulated by addition of enzymes or hydrolysed starter cells to cheese milk.
- ❑ Ripening of Emmental cheese has been accelerated by using starters grown to high cell numbers in media supplemented with protein hydrolysates and metalloproteinase from *Micrococcus caseolyticus*.

Modified starter cultures

- Addition of modified/attenuated starter culture is to increase number of starter cells without causing detrimental effect on acidification schedule during manufacture, so that the cells contribute only to proteolysis and other changes during ripening.
- Modified starter cultures with attenuated acid producing abilities are added with normal starter cultures during cheese manufacture.
- Selection of starter strains with enhanced peptidase activity provide a more balanced flavor.

Heat and freeze shock treated cells

- ❑ Mixed strain starters or *L. helveticus* culture subjected to various heat-shock treatments reduce their acid producing ability and enhance their rate of autolysis.
- ❑ Combination of neutral proteinase with heat shocked *L. helveticus* to final level of 4×10^6 cfu/g curd also accelerated the ripening of cheese.
- ❑ Addition of heat-shocked lactobacilli increased peptidolysis and produce good flavor in low-fat semi-hard cheese.
- ❑ Acid-producing ability of LAB can be reduced by sub-lethal heat treatment while slightly reducing enzyme activities. Heating at 59 or 69°C for 15 s was optimal for mixed mesophilic and thermophilic lactobacilli cultures respectively.
- ❑ 90% of heat-shocked cells added to cheese milk at a level of 2% (v/v) were entrapped in curd.

Lysozyme treated cells

- Addition of lysozyme-sensitized cells to cheese milk at level of equivalent to 10^{10} cells/g of cheese indicated that intracellular dipeptidases were released and concentration of free amino acids significantly increased.
- No effect on rate of flavor development.
- Lysozyme treated cells may not be viable for large -scale cheese manufacture owing to cost of enzyme.

Mutant starter cultures

- Use of lactose negative mutants, which do not affect the rate of acid development but provide additional enzymes.**
- Lac-mutant of starter strains provide packets of uniformly distributed proteinases/peptidases, enhancing the production of peptides and free amino acids without interfering with acid production during manufacture.**

Use of high pressure treatment

HPT is a food processing technology primarily due to increasing interest in preservation of foods.

Applying high pressure to food products modify interactions between individual components, influences rate of enzymatic reactions and can inactivate microorganism.

Application of HPT to cheese results in increase in moisture content and pH and cause changes to cheese matrix and lysis of cells, which contribute to ripening.

HPT affects pattern of proteolysis during ripening, effect is dependent on type of cheese, duration and temperature of pressure treatment.

Advantages in cheese manufacture and ripening.