Enzymes in milk and dairy products

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Biochemical reactions in dairy products

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Enzymes in milk and dairy products

Enzymes originating from:

(i) Indigenous enzymes of milk

(ii) Coagulant,

(iii) Microbial enzymes (starter LAB, secondary/adjunct culture, and NSLAB)

(vi) Exogenous enzymes.

Indigenous (Native) Milk Enzymes

- These enzymes originate mainly from leakage of blood into the milk or immune system components.

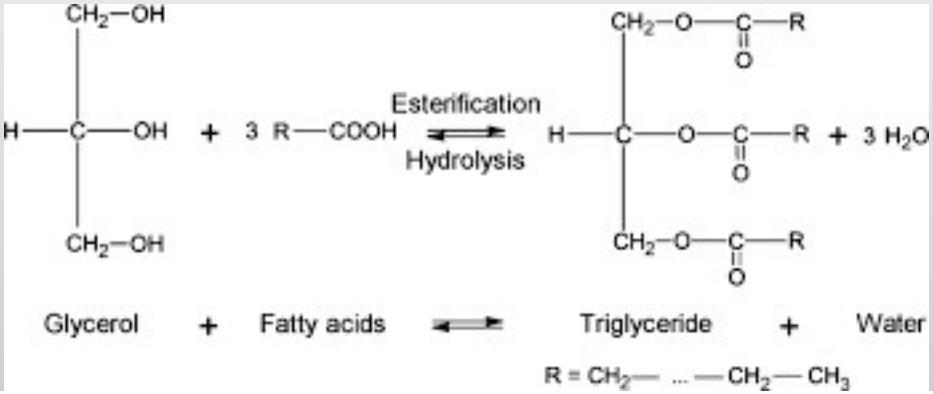
1. **Proteases:** Plasmin (milk alkaline protease) and cathepsin D (milk acid proteinase)
2. **Lipases:** Lipoprotein lipase (LPL)

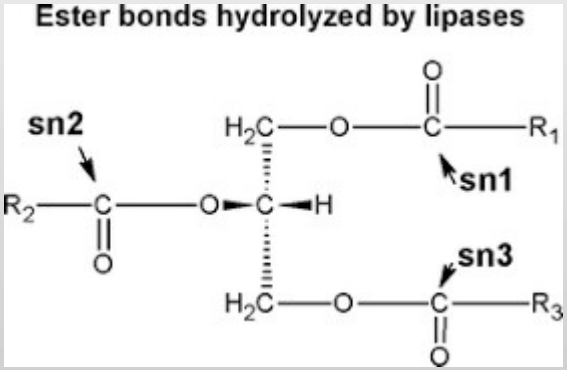
Native Proteases

- The hydrolysis of milk proteins by proteases affects the texture and flavor of dairy products.
- This can have either beneficial or detrimental effects, depending on the extent of hydrolysis and type of dairy product.
- Plasmin occurs in milk along with its precursor plasminogen (inactive form of plasmin) and is associated with the casein micelles.
- Plasmin and cathepsin D act on β- and αS1-casein, respectively.
- Cathepsin D originates from the lysosomes of somatic cells.

Native Lipases

- Lipoprotein lipase (LPL) is the indigenous milk lipase that originates from blood.
- Contributes to the lipolysis and off-flavor formation in dairy products. LPL is rather nonspecific for the type of fatty acid but is specific for sn1 and sn3 positions of mono-, di- and triglycerides.
- Lipolysis in milk leads to preferential release of short- and medium-chain fatty acids, which in milk triglycerides are esterified predominantly at the sn3 position.





2. Coagulant

- Coagulants are milk clotting enzymes.
- The ready-to-use forms of these enzymes are known as rennet .
- Milk clotting enzymes such as chymosin and pepsin are able to hydrolyze peptide bonds and are responsible for the coagulation during cheese production by the specific cleavage of the Phe105-Met106 bond of κ-casein, which destabilizes the casein micelle structure .
- Enzymatic milk coagulation is a two-phase process.
- In the first phase, the enzyme hydrolyzes the κ-casein molecule and splits the protein into two fragments: hydrophobic para-κ-casein and a hydrophilic macropeptide. The second phase consists of the coagulation of the casein micelles that have been destabilized by proteolytic hydrolysis

Other than chymosin from mammalian species (mainly bovine), there are also commercially available enzymes produced by *Rhizomucor* spp., which have gained wide industrial acceptance as substitutes for the bovine chymosin.

However, it is known that fungal rennet preparations may present a degree of residual proteolytic activity that subsequently participates in proteolysis of the caseins during ripening and results in the production of bitter peptides during cheese ripening.

- Indigenous enzymes and coagulants are not stable against heat treatments.
- Activity of LPL in raw milk cheeses was extensively inactivated at 72°C for 15 seconds.
- Rennet enzymes can also be inactivated at the curdling stage and are inhibited by added salt.
- Residual chymosin activity was known as decreased during ripening

Proteases

- Proteases can be divided into four classes:

-cysteine proteases,
-serine proteases,
-aspartic proteases and
-metalloproteases.

Each class has a characteristic set of functional amino acid residues arranged in a particular configuration to form the active site.

Proteases can also be subdivided into two major groups based on their ability to cleave N- or C-terminal peptide bonds (exopeptidase) or internal peptide bonds (endopeptidase)

Aspartic (acid) protease, having an endopeptidase property with two aspartic acid residues, is the most important protease group in cheese technology because of its milk clotting effect during cheese production

3.Enzymes From Microbial Sources

-Enzymes of Starter LAB and NSLAB
-Enzymes of Secondary Cultures
-Exogenous (Adjunct) cultures

Enzymes of Starter LAB and NSLAB

- The starter LAB reach maximum numbers shortly after cheese production, followed by a gradual decrease during ripening as a consequence of lysis.
- In contrast, NSLAB are typically present at low numbers in the beginning of the cheese production process, but increase during the months of ripening and ultimately dominate the microbiota of long-ripened cheeses.
- The proteases in LAB are anchored to the cell membrane and protrude through the cell wall. The proteolytic system of LAB is described as cell envelope-associated proteases (CEPs) and are responsible for the formation of many small peptides in cheese and the release of amino acids.
- In most cheese varieties, LAB are the major contributors to flavor development through the action of their intracellular enzyme systems .

Enzymes of Secondary Cultures

- Most cheese varieties, particularly traditional cheeses made from raw milk, have a secondary part in their microbiota which is important for functionalities other than acid production.
- Among fungi, Penicillia are good producers of extracellular enzymes, such as lipases and proteases .
- Extracellular and intracellular aminopeptidases purified from Brevibacterium linens are particularly active on αS1- and β- caseins.
- Pseudomonas spp. is another bacterial strain that was reported to have proteolytic activity.
- Propionibacterium spp. are weakly proteolytic but strongly peptidolytic.

Exogenous (Adjunct) Enzymes

- The main aim of employing exogenous enzymes in cheese industry is to accelerate cheese ripening. Furthermore, the use of these enzymes provides high organoleptic properties.
- Commercial protease and lipase preparations can be added to milk or cheese directly to produce the relevant enzymes.
- In contrast to NSLAB, adjuncts are specifically selected and intentionally added to cheese or milk to balance then textural and flavor profiles that are otherwise hard to attain.
- Despite their positive effects, addition of these enzymes can also stimulate the bitter taste in cheese.

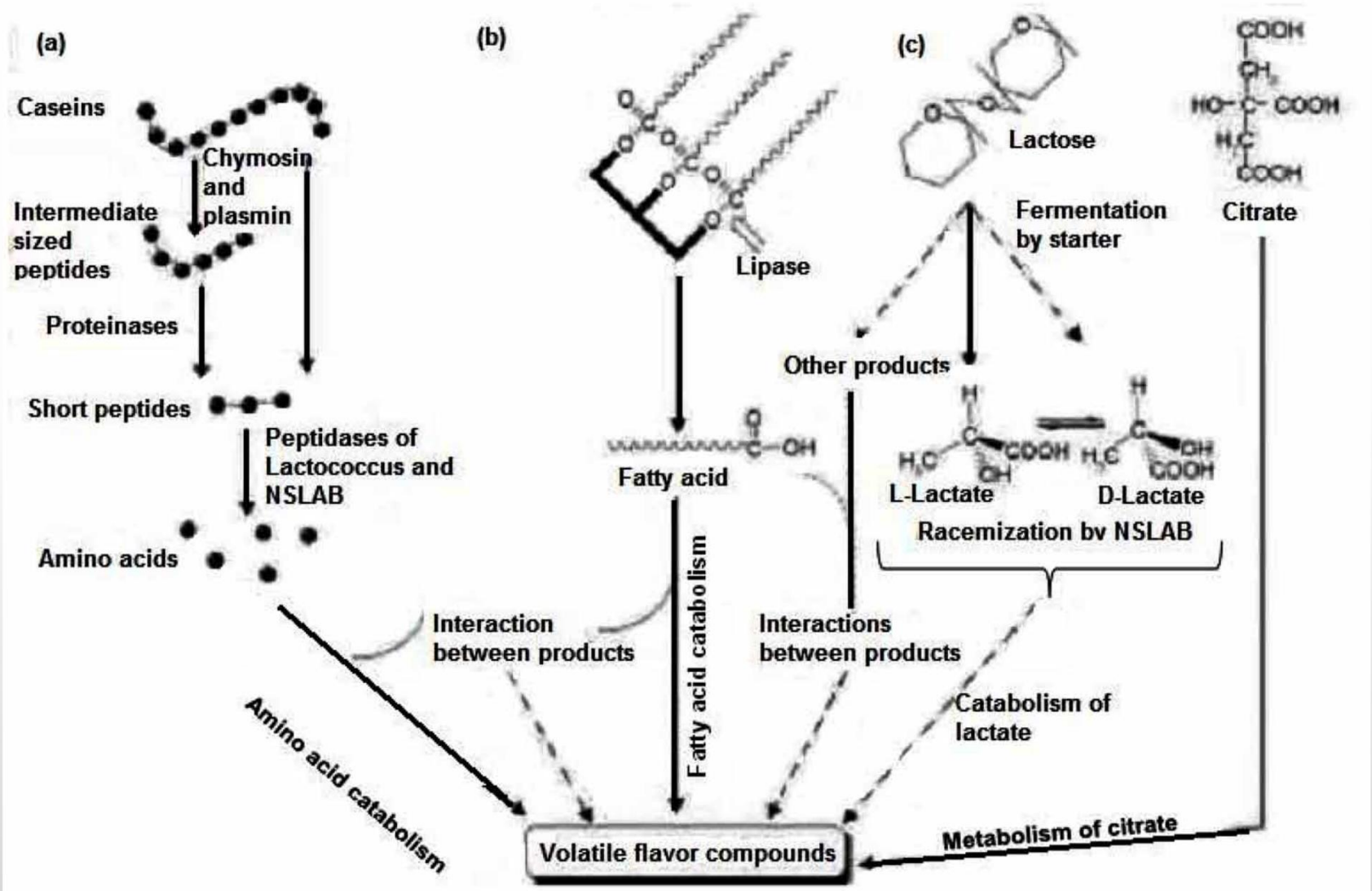
BIOCHEMICAL EVENTS OCCURRED BY ENZYMATICAL REACTIONS

1. Primary Events

→ Glycolysis
→ Lipolysis
→ Proteolysis

2. Secondary Events

→ Catabolism of fatty acids
→ Catabolism of amino acids
→ Lactate and citrate metabolism


(a)
Caseins
Chymosin and plasmin
Intermediate sized peptides
Proteinases
Short peptides
Peptidases of Lactococcus and NSLAB
Amino acids
(b)
Lipase
Fatty acid
(c)
Lactose
Fermentation by starter
Other products
L-Lactate
D-Lactate
Racemization by NSLAB
Citrate
Interaction between products
Fatty acid catabolism
Interactions between products
Catabolism of lactate
Amino acid catabolism
Volatile flavor compounds
Metabolism of citrate

Primary events

- The biochemical alterations in cheese during ripening are grouped into two: primary events and secondary events.
- Primary events include the metabolism of residual lactose (glycolysis) and of lactate and citrate, and breakdown of fat (lipolysis) and breakdown of protein (proteolysis).
- Lactose is broken down to glucose and galactose.
- Glucose is efficiently used for the production of ATP and lactate, whereas galactose accumulates in dairy products due to partial lactose fermentation by lactic acid bacteria..

Secondary events

Following the primary events, secondary events occur including; the catabolism of fatty acids and amino acids.

Products of secondary biochemical events directly influence the development of many volatile flavor compounds

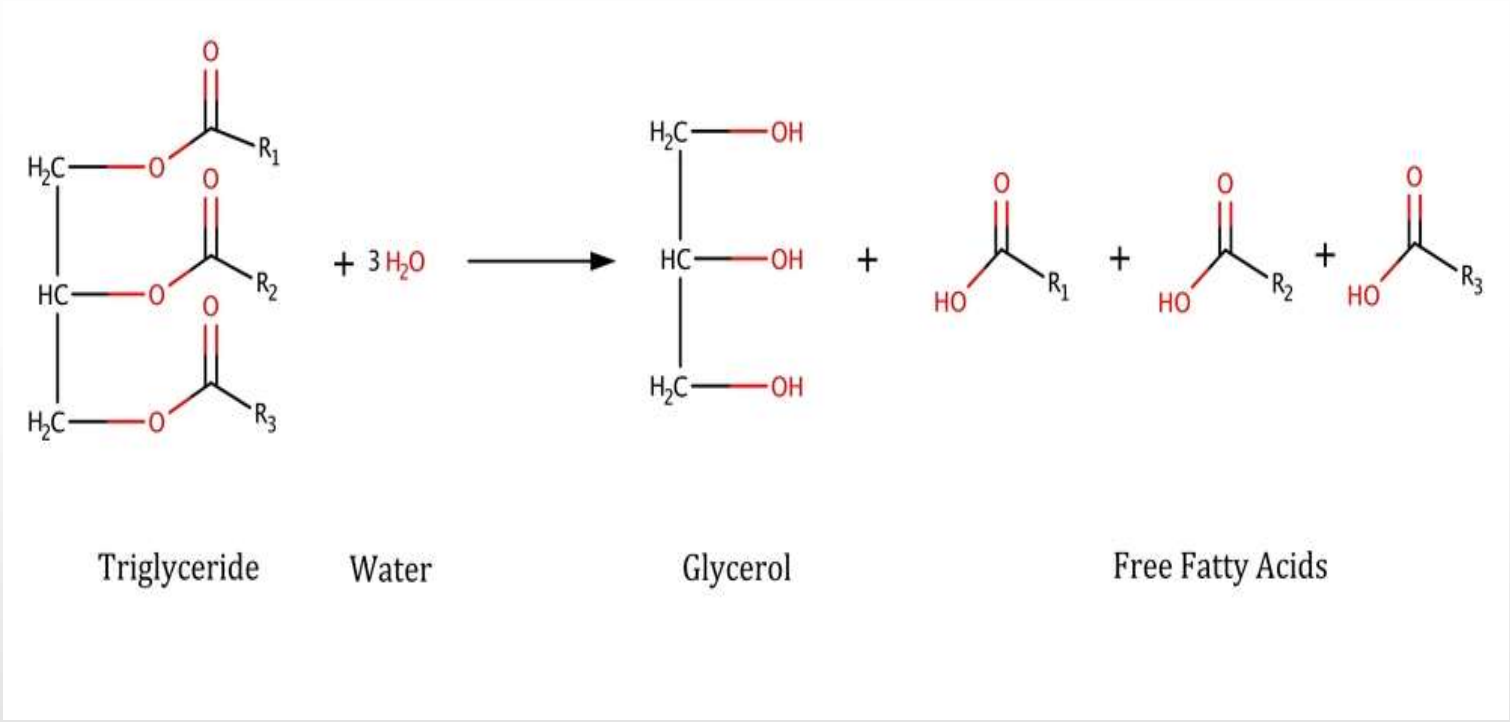
Glycolysis

- Glycolysis includes the fermentation of lactose to lactic acid by LAB and it is an essential primary reaction in the manufacture of all dairy products.
- Glycolysis is terminated with the transition of lactose to lactic acid, (salt is an inhibitory effect in cheese).
- During lactose metabolism the largest proportion of lactose (98%) in milk is removed with separated whey during cheese production
- The three main metabolic processes that occur during glycolysis are lactose, lactic acid (lactate) and citrate metabolisms.

Glycolysis

- The secondary microbiota that colonize and dominate the cheeses rapidly metabolize lactate to CO2 and H2O and cause an increase in pH. When lactate is exhausted, secondary cultures metabolize the proteins, producing NH3, which diffuses inside the cheese and increase the pH. The elevated pH stimulates the activity of native or microbial proteases which contributes to proteolysis in the further stages.
- Citrate metabolism is performed by (Cit+) microorganisms and mainly important for Dutch cheese with holes (eyes). The citrate found in milk is approximately 1.8 g/L and 94% of it is in soluble form, which is also lost with whey during production. The remaining 6% is in colloidal form, and is metabolized to metabolic products such as acetic acid, diacetyl and CO2 by Leuconostoc spp. and (Cit+) Lactococcus spp.

Lipolysis



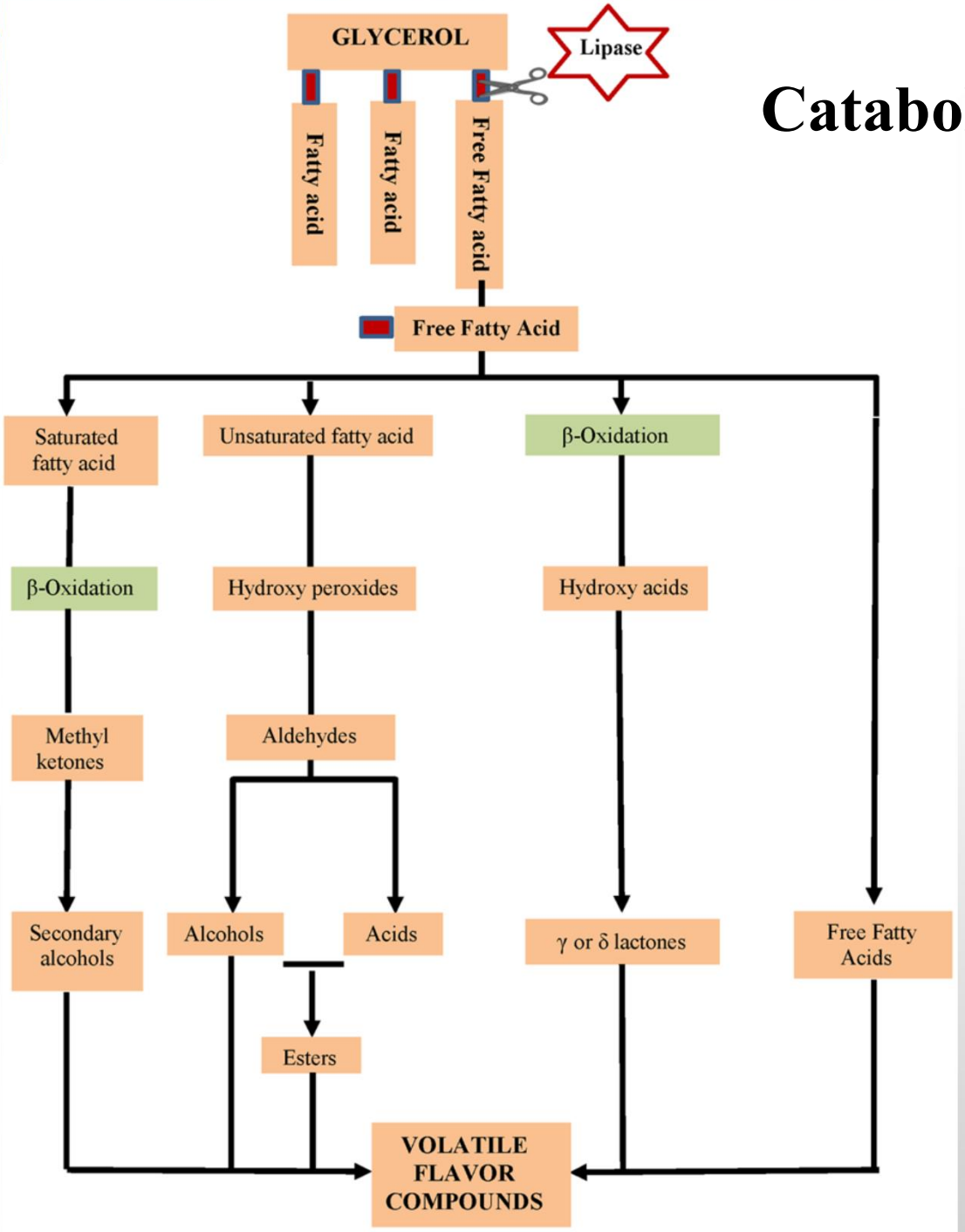
Lipolysis

- Lipolysis is defined as the hydrolysis of ester bonds in triglycerides, resulting in the generation of fatty acids and glycerol.
- The release of free fatty acids (FFA) is catalyzed by lipases, which could be residual native milk lipoprotein lipase (LPL), rennet preparations containing pregastric esterase (PGE), lipase and esterase of starter LAB and NSLAB, adjunct cultures or secondary fungal cultures and exogenous lipolytic enzymes.
- Lipolysis and modification of resultant FFAs are significant contributors to overall cheese aroma. However, extensive lipolysis is also considered undesirable in most cheese varieties as it can cause a rancid taste due to an excessive accumulation of volatile FFAs.

Catabolism of Fatty Acids

- FFAs released by lipolysis are used as the precursors for consecutive secondary reactions and are converted to other sapid and aromatic compounds like methyl ketones, lactones and alcohols.
- The concentrations of methyl ketones in Blue cheeses are proportional to the level of lipolysis

Catabolism of Fatty Acids



Catabolism of Fatty Acids

The catabolism of fatty acids in cheese involves 3 main stages:

1. Oxidation of β-ketoacids,
2. Decarboxylation to methyl ketones with one less carbon atom,
3. Reduction of methyl ketones to the corresponding secondary alcohol.

A number of factors affect the rate of methyl ketone production, including temperature, pH, physiological state of the microorganism and the ratio of the fatty acids' concentration to the dry weight of spores.

Both resting spores and fungal mycelium are capable of producing methyl ketones, but the rate of production of methyl ketones does not directly depend on the concentrations of FFA precursors.

Proteolysis

- Proteolysis is the hydrolysis of cheese proteins leading to the formation of medium and small soluble peptides and free amino acids from large water-insoluble peptides (comparable in size to intact caseins).
- It is the most complex of the three primary biochemical events during cheese ripening.
- Proteolysis is widely considered to be the most important process for the development of sensory and textural properties of cheese, except for Blue cheese which is dominated by the mechanism of lipolysis
- It also contributes to off-flavors in cheeses

Proteolysis

Proteolysis is catalyzed by proteases and peptidases from residual coagulant, native milk enzymes (especially plasmin), starter LAB, NSLAB, and in certain varieties other than secondary microbiota.

Particularly, extensive proteolysis occurs in surface mold- and surface smear- ripened cheeses because of the very potent proteolytic system of their secondary cultures

Intracellular and extracellular proteases are available mainly purified from different microorganisms.

Proteolysis

Proteolysis contributes to cheese ripening through **four** pathways:

1. Direct contribution to flavor via the formation of peptides and amino acids or indirect contribution via catabolism of amino acids to amines, acids, thiols, thioesters etc.,

2. Higher release of sapid compounds during mastication,

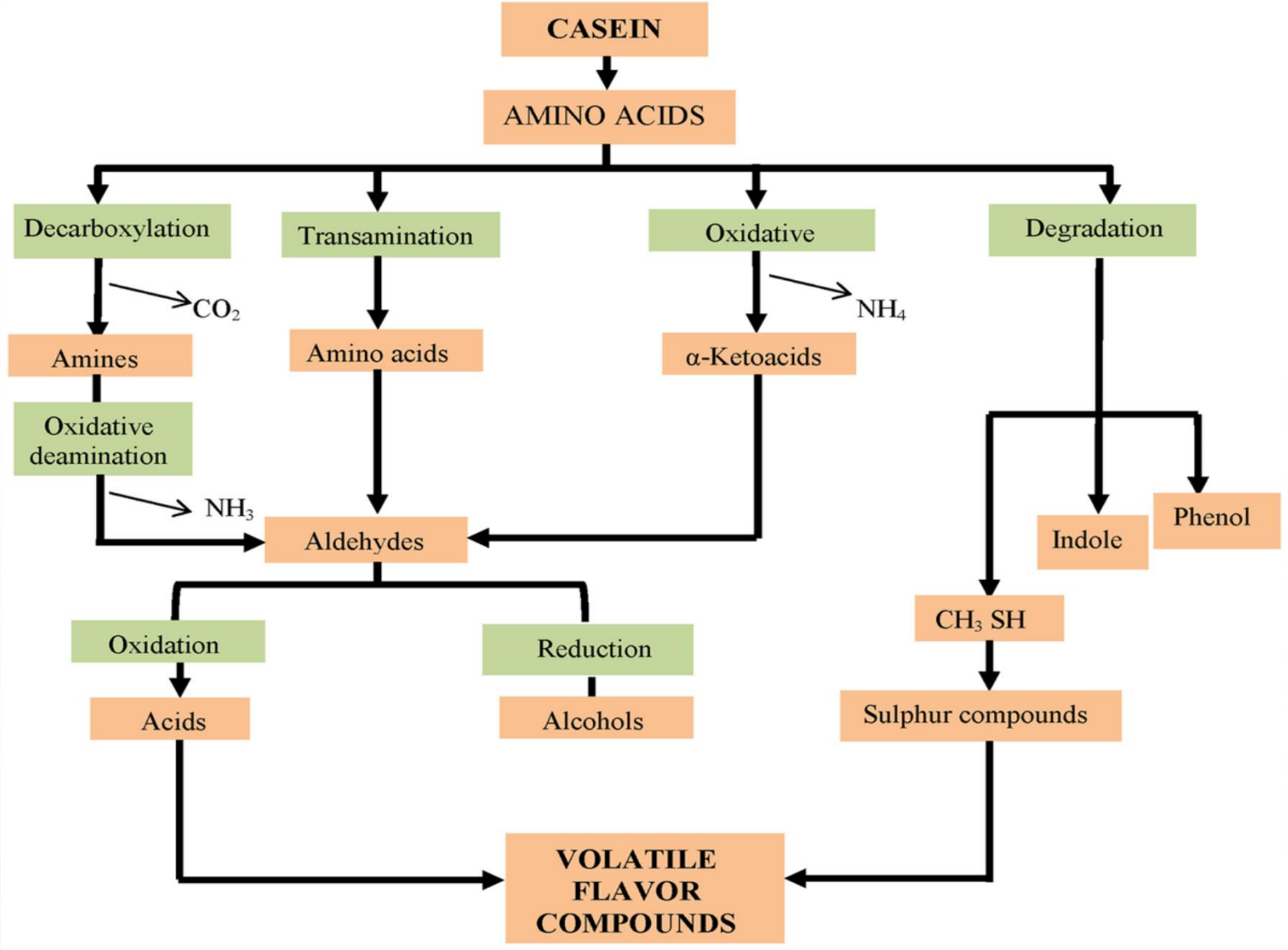
3. Alterations in pH via the formation of NH3,

4. Modifications of the texture of cheese due to the breakdown of the protein network, increase in pH and greater water binding by the newly-formed amino and carboxyl groups

Catabolism of Amino Acids

- Amino acid catabolism, which is particularly significant in mold- and smear ripened cheese varieties, is an indicator of extensive proteolysis.
- Decarboxylation involves the conversion of amino acid to the corresponding amine with loss of CO_2.
- Deamination results in the formation of NH_3 and α-ketoacids.
- Further, transamination results in the formation of other aminoacids by the action of transaminases.
- Aldehydes formed by the above processes can then be oxidized to acids or reduced to the corresponding alcohols.
- In addition, sulphur containing compounds are another volatile group produced from aminoacids.

Catabolism of Amino Acids



Catabolism of Amino Acids

- Wild isolates of *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* strains are reported to be involved in aa catabolism
- Wild strains are not naturally associated with a rich environment such as milk, which makes them more dependent on synthesis of their own amino acids compared to industrial strains.
- The absence of some amino acid biosynthethic pathways in NSLAB and secondary cultures might be a consequence of their adaptation to dairy products.