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Biochemical Processes During Cheese Ripening

Peynir Olgunlaşmasında Biyokimyasal Olaylar

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ABSTRACT

Cheese ripening entails specific biochemical changes that occur under certain conditions during storage. These changes allow different cheese varieties to develop their unique characteristics. The ripening process is influenced by both primary and secondary biochemical events. These are driven by coagulating enzymes, milk's natural enzymes, and the enzymes of both starter and non-starter microflora. The main biochemical events during cheese ripening include proteolysis, lipolysis, and the metabolism of citrate and lactate. Secondary biochemical reactions then process the primary metabolic products, such as lactic acid, fatty acids, and amino acids. This leads to the formation of volatile compounds like alcohols, aldehydes, ketones, acids, lactones, phenols, esters, and sulfur compounds, which play a crucial role in determining the cheese's quality. These processes give each cheese type its distinctive features, like aroma, taste, color, texture, and pore structure, influencing consumer preferences. This review provides insights into the biochemical events that occur during the cheese ripening period.

Keywords: Cheese biochemistry, enzyme activity in cheese, glycolysis, lipolysis, proteolysis, volatile compounds.

ÖZ

Peynir olgunlaşması, depolama sırasında belirli koşullar altında gerçekleşen spesifik biyokimyasal değişimlerdir. Olgunlaşmadaki değişimler, farklı peynir çeşitlerinin kendine özgü özelliklerini geliştirmesine olanak tanımaktadır. Olgunlaşma süreci, birincil ve ikincil biyokimyasal olaylar tarafından şekillendirilmektedir. Bu olaylar, pıhtılaştırıcı enzimler, sütün doğal enzimleri ile starter ve starter olmayan mikrofloranın enzimleri tarafından yönlendirilmektedir. Peynir olgunlaşması sırasında meydana gelen temel biyokimyasal olaylar arasında proteoliz, lipoliz, sitrat ve laktat metabolizması bulunmaktadır. Birincil metabolik ürünler (örn., laktik asit, yağ asitleri ve amino asitler) ikincil biyokimyasal reaksiyonlarla işlenmekte ve bu süreçte alkol, aldehit, keton, asit, lakton, fenol, ester ve sülfür bileşikleri gibi uçucu bileşikler oluşmaktadır. Bu uçucu bileşikler, peynirin kalitesini belirlemede kritik bir rol oynamaktadır. Bu süreçler, her peynir türüne özgü aroma, tat, renk, doku ve gözenek yapısı gibi ayırt edici özellikler kazandırarak tüketici tercihlerini etkilemektedir. Bu derleme, peynir olgunlaşma dönemi boyunca gerçekleşen biyokimyasal olaylara ilişkin bir bakış sunmaktadır.

Anahtar Kelimeler: Glikoliz, lipoliz, proteoliz, peynir biyokimyası, peynirde enzim aktivitesi, uçucu bileşikler.

INTRODUCTION

Milk is frequently transformed into long-lasting dairy products to extend its shelf life and enhance its flavor, aroma, and texture. Chief among these is cheese—one of the oldest foods crafted by humans.[1,2] Globally, cheese stands out as one of the most widely consumed dairy items, offering a staggering variety exceeding 2000 types. Each type possesses distinctive shapes, textures, and flavors.[3] The nuanced differences between these cheese varieties are primarily attributed to their production methods and the composition of the raw milk utilized.[2,4]

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Cheese is best described as a bio-complex ecosystem, populated by diverse microorganisms sourced from raw milk, starter cultures, and adjunct cultures.[5,6] The cheese-making process involves pressing, shaping, and salting curd, which can subsequently be consumed either fresh or after undergoing ripening.[7] What often drives consumer preference for cheese is its taste, texture, and overall visual appeal. These defining characteristics, which dictate the organoleptic quality of cheese, undergo development through biochemical transformations during the ripening phase.[6]

Cheese production, a multifaceted procedure punctuated by various stages and unique biochemical events, ensures the cheese's biochemical structure remains in flux throughout its ripening. This dynamism is steered by both primary and secondary biochemical reactions, influenced by coagulating enzymes, the inherent enzymes of milk, and the combined action of starter and non-starter microflora and their respective microbial enzymes.[3]

Within the ripening landscape of cheese, we observe primary biochemical events like proteolysis, lipolysis, and the metabolism of citrate and lactate.[1,8] The secondary events primarily focus on the catabolism of lactic acid, fatty acids, and amino acids which emerge from the primary metabolic activities. These events culminate in the creation of a range of volatile compounds—alcohols, aldehydes, ketones, acids, lactones, phenols, esters, and sulfur compounds—to name a few. It's these compounds that play a decisive role in determining cheese quality and shaping consumer inclinations, endowing each cheese variety with its characteristic aroma, taste, color, and texture.[3,4,9,10] In this review, we discuss the biochemical changes that occur during the cheese ripening process.

1. Ripening in Cheese

Cheese ripening is a crucial technological process rooted in microbiological and biochemical principles. It defines the organoleptic quality of cheese.[5] Ripening involves biochemical transformations, such as glycolysis, proteolysis, and lipolysis, occurring under specific storage conditions, which allows cheese varieties to develop their distinct characteristics, including aroma, taste, color, texture, and porosity.[3,11,12] The biochemical events during cheese ripening are illustrated in Figure 1. Owing to various enzymes derived from milk (like plasmin and lipoprotein lipase), coagulating enzymes (like chymosin), and microorganisms (like lactase and proteinase) active during this period, each cheese type establishes its unique properties.[1,5,13,14]

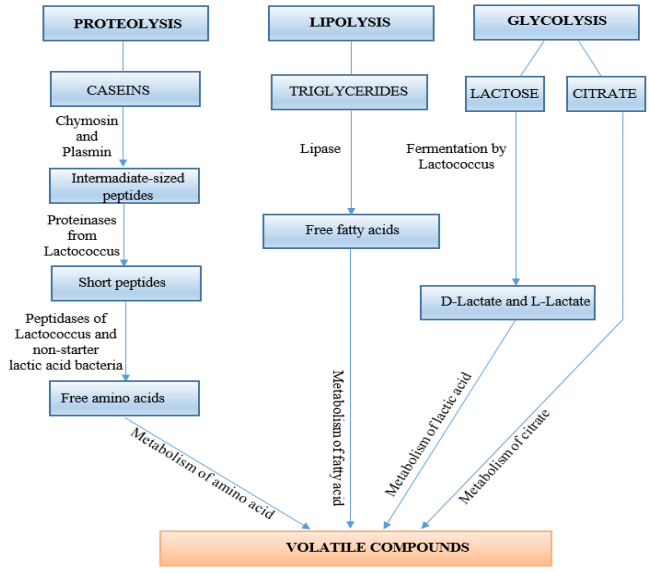

Figure 1. Illustration of primary and secondary biochemical processes during cheese ripening.[10]

The microflora of cheese significantly influences its ripening. The combined metabolic activities of microbial flora on cheese's fat, protein, and carbohydrates refine its organoleptic properties, especially its flavor.[15] This is achieved through a myriad of enzymatic and chemical changes within the cheese matrix. Cheese microorganisms are categorized as starter lactic acid bacteria (primary microflora) and non-starter microorganisms (secondary microflora). In the early cheese production stages, primary microflora, particularly starter lactic acid bacteria, intensifies the acidity by metabolizing lactose.[16-18] This lactic acid production reduces the pH, facilitating clot formation and inhibiting pathogen growth. Yet, during ripening, these bacteria diminish due to autolysis, releasing intracellular enzymes and generating compounds conducive for non-starter lactic acid bacteria (NSLAB) growth.[6,15,18,19]

Frequently utilized starter lactic acid bacteria in cheese-making include species from the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*.[20] These bacteria can be either mesophilic (optimal growth at 25-30 °C) or thermophilic (optimal growth at 40-45 °C).[15] Essential starter cultures in cheese biochemistry are detailed in Table 1.[21]

Table 1. Starter cultures utilized in the production of selected cheeses.[21]

Microorganisms	Types of Cheese Used
Mesophilic group	
Lc. lactis subsp. lactis Lc. lactis subsp. cremoris Lc. lactis subsp. diacetylactis Leu. cremoris	Brie, Camembert, Cheddar, Cottage, Cream Cheese, Danish Blue, Edam, Turkish White Cheese, Gouda, Stilton
Propionobacterium spp.	Emmental, Gruyere
Thermophilic group	
Str. thermophilus Lb. delbrueckii subsp. bulgaricus Lb. helveticus, Lb. lactis	Emmental, Gruyere, Parmesan, Romano
Molds	
P. camemberti	Brie, Camembert
P. roqueforti	Danish Blue, Roquefort, Stilton

Lc: Lactococcus, Leu: Leuconostoc, Str: Streptococcus, Lb: Lactobacillus, P: Penicillium

Contrarily, secondary microflora contribute minimally to acid production. These microorganisms significantly influence the emergence of various volatile compounds (like organic acids and aldehydes) during cheese ripening. Comprising NSLAB, propionic acid bacteria, yeasts, and molds, the secondary microflora predominantly originates either from cheese components or environmental exposure during manufacturing.[16,22] NSLAB significantly enhance the flavor, texture, nutritional value, and microbial safety of many ripened cheeses.[23,24] Nonetheless, some quality issues, particularly off-flavors in later ripening stages, can be attributed to these bacteria.[6] While mesophilic lactobacilli dominate NSLAB in cheese, species from *Pediococcus*, *Enterococcus*, and *Leuconostoc* genera are also present. Predominant mesophilic lactobacilli species in cheese reportedly include *Lb. casei*, *Lb. rhamnosus*, *Lb. plantarum*, *Lb. paracasei*, and *Lb. curvatus*.[20,22]

During the ripening process of cheese, biochemical reactions can be broadly categorized into primary and secondary types. Primary reactions involve the metabolism of milk casein into peptides and amino acids, the conversion of lactose to lactic acid, and the breakdown of fats (triglycerides) into fatty acids. Secondary reactions result in the formation of amines, aldehydes, sulfur compounds, and CO_2 from amino acids and organic acids, with fatty acids further metabolized into secondary alcohols.[8,25,26] Proteolysis, lipolysis, and glycolysis are fundamental biochemical reactions during ripening, playing pivotal roles in producing volatile compounds that influence the cheese's quality, especially its flavor development.[3,5]

2. Primary Biochemical Events

2.1. Glycolysis in Cheese

During cheese production, approximately 96% of the milk's lactose remains in the whey.[1] As cheese undergoes fermentation, the residual lactose in the curd is converted to lactate by lactic acid bacteria. This conversion is fundamental to the creation of all cheese varieties.[13,18,27] Initially, starter lactic acid bacteria metabolize the lactose to lactate through glycolysis. This lactate is subsequently transformed into volatile compounds by NSLAB.[5,6] In certain cheese types, like cheddar, lactic acid formation predominantly occurs in the vat before molding the curds. However, for the majority of cheese varieties, this process transpires after the curds have been set in the molds. Typically, the pH of the curd drops to a range of 5.0–5.3 within approximately 12 hours from the onset of cheesemaking.[28]

Glycolysis, driven by the breakdown of lactate and citrate, is completed during the early phases of the ripening period, typically within the first one or two weeks.[26] The metabolism of the residual lactose in the curd from the cheese production process elevates the acidity, influencing the pH of the cheese.[6,18] The lactic acid produced not only inhibits the growth of undesirable microflora, enhancing cheese quality, but also impacts the texture by affecting the demineralization and solubility of caseins. As a result, cheeses with a higher pH tend to be softer than those with pronounced acidity.[1,6,27,28] Pyruvate, a by-product of lactose metabolism, serves as a precursor for the synthesis of short-chain flavor compounds like acetate, acetoin, diacetyl, ethanol, and acetaldehyde.[5] The final stage of lactose glycolysis involves converting pyruvate to lactate, a reaction facilitated by lactate dehydrogenase.[29]

2.2. Lipolysis in Cheese

Lipolysis plays a crucial role in shaping the flavor and texture of cheese. The fat involved in cheese lipolysis directly adds to its flavor through components like fatty acids or indirectly via the transformation of these acids into volatile compounds, such as methyl ketones and esters.[3,9] Although lipids in foods can degrade through hydrolytic or oxidative processes, cheese experiences limited oxidative changes due to its low oxidation/reduction potential (around -250 mV) and the presence of antioxidants.[6,30] In every cheese variety, triglycerides undergo decomposition into free fatty acids during ripening, courtesy of bacterial and native milk enzymes, including lipases.[5,27,31] These free fatty acids act as precursors for catabolic reactions, resulting in the creation of compounds like methyl ketones,

lactones, esters, alkanes, and secondary alcohols.[30,32]

The sources of lipolytic enzymes in cheese are primarily milk, coagulant (rennet), and the microflora of the cheese, both starter and non-starter types.[1,27] Their action on triglycerides leads to the generation of both medium-chain (with a carbon chain length ≤10) and long-chain (carbon chain length >10) free fatty acids.[30,33] Cheeses derived from pasteurized milk typically lack potent lipolytic enzymes compared to their raw milk counterparts. However, lipolysis still occurs during the ripening phase due to the influence of enzymes from both starter and non-starter microflora.[29]

Lactic acid bacteria, used as a starter culture, generally exhibit a mild lipolytic effect. Most fatty acids result from the breakdown of triglycerides by molds.[1,6,9] The extent of lipolysis varies across cheese varieties. In certain cheeses like cheddar, gouda, and Swiss types, even a moderate presence of free fatty acids can introduce a bitterness, which consumers might interpret as spoilage.[5] Conversely, lipolysis is both necessary and desired for the flavor development in hard Italian cheeses, as well as in blue, camembert, and feta cheeses.[27,32] The flavor contributions from free fatty acids in cheese are largely influenced by pH. At elevated pH levels, free fatty acids, perceived as less aromatic, can often come across as "soapy" since they transform into non-volatile salts. In contrast, at lower pH levels, the free fatty acids remain unbound and high concentrations evoke a sour taste.[12,33,34]

2.3. Proteolysis in Cheese

Proteolysis stands out as the most intricate and pivotal primary biochemical event during cheese ripening. The pH, during cheese ripening, plays a pivotal role in shaping its texture and flavor.[1,25,35] Texture development in cheese due to proteolysis arises from the hydrolysis of its protein matrix, increased water-binding capacity by newly formed carboxylic acid and amino groups through peptide bond hydrolysis, and a decrease in water activity (aw). Furthermore, ammonia, a by-product of amino acid catabolism, elevates the cheese's pH and aids texture development.[36,37] Casein metabolization leads to textural transformations in cheese, turning a rubbery hard curd into a creamy, smooth texture.[5]

Various enzymes, like proteinases and peptidases, facilitate proteolysis during cheese ripening. They have multiple origins: coagulants (e.g., chymosin/rennin), milk (e.g., plasmin, cathepsin D), starter lactic acid bacteria, NSLAB, and secondary starters. For instance, *P. roqueforti* is found in blue cheese, while *P. camemberti* is present in camembert cheese. In some instances, to hasten ripening, exogenous proteinases or intracellular peptidases may be added to milk or curd.[1,8,36] Two primary sources of proteolytic enzymes in cheese are chymosin (rennin), a residual coagulant left in curd post-whey filtration, and plasmin, which transfers from blood to milk.[5,31]

Proteolysis in cheese is twofold:

i. In primary proteolysis, chymosin and plasmin hydrolyze caseins, yielding medium-sized peptides.[35]

ii. Secondary proteolysis results in further breakdown into smaller peptides and amino acids due to hydrolysis by proteinases and peptidases released from the breakdown of starter lactic acid bacteria and NSLAB.[8,25,37,38]

The peptides and amino acids derived from the cheese matrix via proteolysis serve as critical substrates for various catabolic reactions, producing significant flavor and aroma compounds, such as amines, aldehydes, alcohols, acids, phenols, and sulfur compounds.[3,39] Since each cheese variety has distinct production and ripening conditions, their proteolysis methods can differ. Factors influencing proteolysis in cheese include ripening temperature and duration, pH, moisture content, residual coagulant activity, the transformation of plasminogen to plasmin, and the growth of secondary microflora.[3,29]

3. Secondary Biochemical Events

3.1. Lactate and Citrate Catabolism

Lactose is initially converted into glucose and galactose by the lactase enzyme (β-galactosidase) produced by lactic acid bacteria (LAB). The homofermentative LAB species, such as *Lb. acidophilus*, *Lb. bulgaricus*, and *Lb. helveticus*, possess aldolase and hexoisomerase enzymes but lack phosphoketolase. These homofermentative LAB utilize the Embden-Meyerhof Parnas (EMP) fructose-1,6 diphosphate metabolic pathway, also known as the glycolytic pathway. Here, glucose is first transformed into pyruvic acid and then predominantly (about 90%) into lactic acid.[6,18,21,40,41]

On the other hand, heterofermentative LAB like *Leuconostoc* spp., *Lb. casei* group, and *Lb. plantarum* are equipped with the enzyme phosphoketolase. These LAB species produce not only lactic acid (around 50%) but also

acetic acid, ethyl alcohol, and CO_2 from glucose, using the phosphoketolase (phospholytic) pathway.[6,21,40,41]

Lactate metabolism plays a vital role in shaping the organoleptic properties of aged cheeses, such as camembert and brie.[21,29] Figure 2 provides a schematic illustration of the biochemical pathways of lactose and citrate catabolism, which lead to the generation of flavor compounds by LAB in cheese.

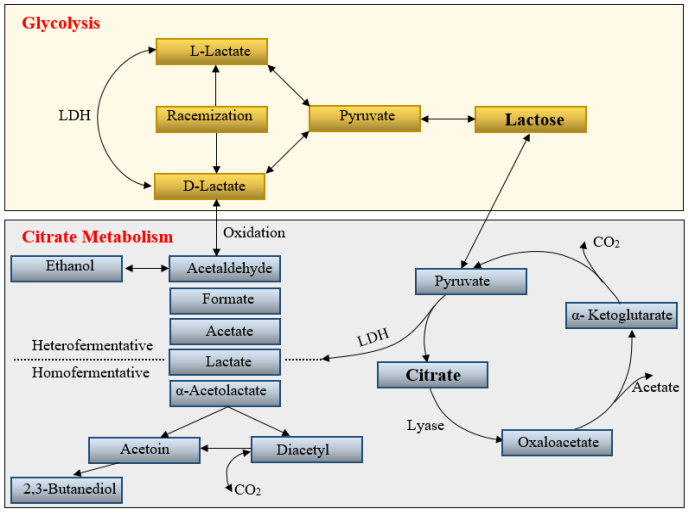

Figure 2. Diagram illustrating the catabolism of lactose and citrate by lactic acid bacteria in cheese.[15,41]

Lactate and citrate serve as significant substrates for various reactions that take place during cheese ripening and the formation of flavor. The metabolic pathways of lactate in cheese include:[1,5,28]

i. In most cheeses, NSLAB racemizes L-lactate to D-lactate (see Fig. 2). Additionally, *Lactobacillus* spp. can produce D-lactate from any residual lactose. While racemization does not influence flavor, calcium-D-lactate, being less soluble than L-lactate, results in calcium lactate pentahydrate crystals that appear as white spots on cheese surfaces.

ii. In Swiss-type cheeses, *Propionibacterium freudenreichii* catabolizes lactate, producing by-products like H_2O, CO_2, propionate, and acetate, leading to the formation of characteristic pores.

iii. *P. camemberti* breaks down lactate into CO_2 and H_2O, crucial for tissue development in surface-ripened cheeses such as camembert and brie.

iv. Some NSLAB (notably *Pediococcus* spp.) can oxidize lactate to formate, ethanol, and acetate in the presence of O_2. This lactate oxidation depends on NSLAB population and the available O_2, determined by the packaging material's oxygen permeability.

v. Anaerobic lactate metabolism by *Clostridium tyrobutyricum*, leading to the production of butyrate, CO_2, and H_2, can result in the “late swelling” defect in cheese-making.

Around 90% of milk's citrate content is soluble and is largely lost with the whey. Metabolizing the minor amounts of citrate remaining post cheese production often results in the formation of organoleptic properties. This process enhances aroma compounds and the creation of cheese pores.[42] Initially, citrate lyase hydrolyzes citrate into oxaloacetate and acetate (refer to Figure 2). Subsequently, oxaloacetate decarboxylates to pyruvate, giving rise to compounds like diacetyl, acetoin, and 2,3-butanediol.[41]

While *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* do not metabolize citrate, *Lc. lactis* ssp. biovar *diacetylactis* and *Leuconostoc* spp. do so, producing favorable flavor compounds like diacetyl, acetoin, and 2,3-butanediol.[27,34,43] Some facultative heterofermentative *Lactobacillus* species, such as *Lb. casei* and *Lb. plantarum*, synthesize citrate into acetate, diacetyl, and acetoin. *Lactococcus* spp. and *Leuconostoc* spp. exhibit similar catabolism.[15,28] Moreover, both *Enterococcus* spp. and *Weissella* spp. can metabolize citrate. The acetoin/diacetyl catabolism pathway of citrate, depicted in Fig. 2, is essential to produce the volatile compounds granting cheese its distinctive buttery taste.[42] The CO_2 emerging from the metabolism of lactate and citrate forms the signature pores in cheeses like emmental and cottage Dutch types. However, in solid cheeses, this feature is undesirable.[5,34]

3.2. Catabolism of Free Fatty Acids

Free Fatty Acids (FFA), particularly acetic, octanoic, and decanoic acids, directly infuse flavor into cheese. Yet, the volatile by-products of their catabolism, such as methyl ketones, secondary alcohols, straight-chain aldehydes, lactones, and esters, have a profound impact on cheese flavor.[3,27] Figure 3 schematically illustrates the biochemical pathways of fatty acid catabolism leading to flavor compound formation in cheese.

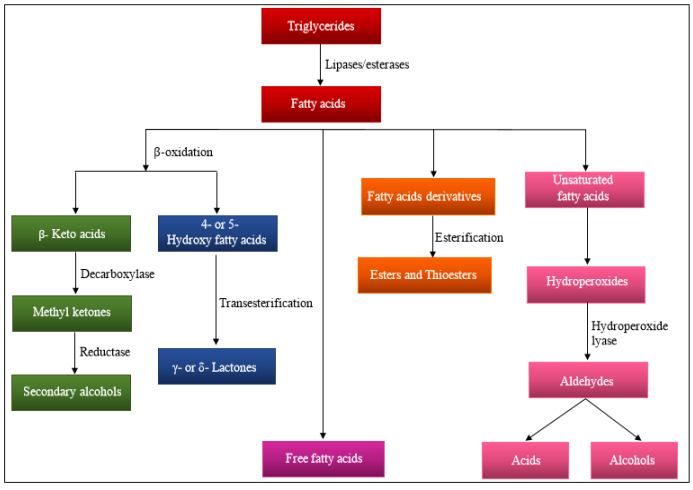

Figure 3. Biochemical pathways of fatty acid catabolism in cheese: A schematic representation.[6,34]

Esters in many cheese varieties result from the reaction between a fatty acid and an alcohol. In cheese, the most prevalent esters are ethyl esters of straight-chain free fatty acids (C2:0–C10:0), with ethanol—derived from lactose or amino acid catabolism—being the most common alcohol forming methyl, propyl, and butyl esters.[30] Lactones, cyclic compounds, originate from hydroxyacids via intramolecular esterification. Cheese contains both γ- and δ-lactones, with their production during ripening constrained by the availability of hydroxyacids, their precursors. Moreover, free fatty acids can be transformed into methyl ketones, crucial to the flavor of blue-veined cheeses, particularly those surface-ripened by molds like *Penicillium* spp., through β-oxidation.[27,29,33,34]

Typically, secondary alcohols and even-numbered methyl ketones arise from the autoxidation of unsaturated fatty acids. Singular chain methyl ketones and secondary alcohols form from the β-oxidation of free fatty acids (as depicted in Figure 3). In cheeses, enzymes such as lipoxygenase and hydroperoxide lyase from *P. camemberti* can also generate secondary alcohols by reducing methyl ketones.[30] Although numerous aldehydes emerge from amino acid catabolism, straight-chain aldehydes, like butanal and heptanal, might arise from the oxidation of unsaturated fatty acids. However, the oxidation extent in cheese remains minimal due to its low redox potential and inherent antioxidants.[34]

3.3. Catabolism of Free Amino Acids

Free amino acid catabolism significantly affects the development of cheese's flavor and textural attributes.[27] These amino acids undergo various reactions—such as deamination, amination, and decarboxylation—resulting in volatile and non-volatile compounds.[3,44] Primary products of this catabolism include aldehydes, alcohols, carboxylic acids, amines, and sulfur compounds.[6,45] A schematic showcasing the biochemical pathways of amino acid catabolism that leads to flavor compounds in cheese can be seen in Figure 4.

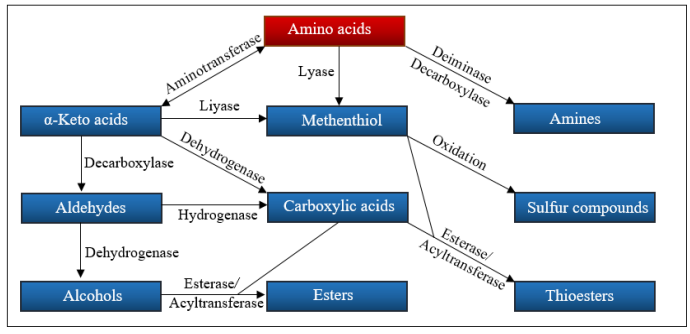

Figure 4. Schematic representation of the biochemical pathways of amino acid catabolism in cheese.[34,44]

Sulfur-containing aromatic compounds, such as dimethyl sulfide, primarily arise from methionine degradation, contributing to the characteristic "garlic" flavor found in cheeses like cheddar and camembert.[3,4,15,46,47] Another pivotal pathway in free amino acid catabolism involves the transamination reaction, central to the breakdown of all amino acids by lactic acid bacteria.[27,46] This reaction transforms aromatic amino acids into α-ketoacids.[5,6,33,34] Aminotransferases, intracellular enzymes present in starter cultures, facilitate this reaction, needing pyridoxal-5-phosphate for their function.[1,44] Resulting α-ketoacids, acting as intermediaries, are reduced by the cheese microflora into various compounds in the ongoing biochemical process.[5,44,45]

There is a symbiotic relationship between starter lactic acid bacteria and NSLAB in the aroma formation of certain cheeses, such as cheddar. For instance, *Lactobacillus* spp. initiates the conversion of amino acids to keto- and hydroxyl acids, whereas *Lactococcus* spp. transforms these by-products into carboxylic acids.[27]

Amino acids undergo deamination and decarboxylation, leading to the production of α-keto acids, ammonia, and amines.[3] These are further transformed into volatile compounds such as alcohols, esters, acids, and aldehydes. Decarboxylation involves the transition of an amino acid to an amine (primarily tyramine) accompanied by a CO_2 loss.[1] Branched-chain amino acids, including leucine, isoleucine, and valine, can be decarboxylated to produce amines with off-putting flavors, for instance, ketoisocaproate, α-keto isovalerate, and α-keto-L-methyl valerate.[5] The deamination reaction produces NH_3, a vital component in certain cheeses like camembert and gruyere. Additionally, ammonia can arise from the oxidative deamination of

aldehyde-producing amines.[1] Ammonium compounds, pivotal for the flavor in mold-ripened cheeses such as camembert and brie, are linked to the pronounced proteolytic activities of the *Penicillium* genus.[6]

CONCLUSION

As cheeses ripening, they develop distinct flavor (taste and aroma), appearance, and texture characteristics due to enzyme-driven biochemical reactions from diverse sources, like milk, coagulants, and microflora. These biochemical transformations can be categorized into primary events (like glycolysis, proteolysis, and lipolysis) and secondary ones (such as the catabolism of lactate, citrate, fatty acids, and amino acids). It is evident that volatile compounds, stemming from secondary biochemical processes, are crucial in establishing the unique organoleptic properties of cheeses. A deeper grasp of cheese biochemistry can greatly influence production and storage conditions, enhancing cheese quality standards. Moreover, fully understanding the mechanisms behind the formation of volatile compounds and aroma identification can offer the cheese industry substantial benefits, paving the way for standardized quality cheese production and innovative manufacturing processes.

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