Cheese: Chemistry and Microbiology

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Introduction

Milk has an average composition that varies depending on the species (human, cow, goat, and buffalo; see Table 1), the breed, the animal’s feed, and the stage of lactation. In cheesemaking, the curds obtained after coagulation of milk will usually undergo significant modifications (Figure 1), depending on the type of cheese. Said modifications are caused by the specific technological procedures, which influence the activity of the microbial flora present throughout the cheese processing, and the enzymes native or added to milk. All together, these factors lead to the final product – cheese – that has a chemical composition quite different from milk (Table 2).

However, for simplification, those transformations may be considered essentially caused by three chemical reactions – proteolysis, lipolysis, and glycolysis – acting on milk major components: proteins, fats (lipids), and sugar (lactose).

Protein and Proteolysis in Cheese

Proteins are chains of amino acid molecules connected by peptide bonds (Figure 2). There are many types of proteins, and each has its own amino acid sequence (typically containing hundreds of amino acids). There are 22 different amino acids of which nine are essential (they cannot be made by the human body), and they can combine to form protein chains.

The amino acids within protein chains can bond across the chain and fold to form three-dimensional structures. Proteins can be relatively straight or form tightly compacted globules or be somewhere in between. The term ‘denatured’ is used when proteins unfold from their native chain or globular shape, due to a physical (heat), chemical (acids), or enzymatic (rennet) action.

On average, cow’s milk contains 3.3% total protein. Milk proteins contain all nine essential amino acids required by humans. The milk proteins can be divided into two main groups: the casein family that contains phosphorus and will coagulate or precipitate at pH 4.6 and the serum (whey) proteins that do not contain phosphorus and remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow’s milk, approximately 82% of milk protein is casein and the remaining 18% is serum or whey protein.

The casein family of protein consists of several types of caseins (α, β, κ), and each has its own amino acid composition, genetic variations, and functional properties. The caseins are suspended in milk in a complex called a micelle (see Figure 3). The high phosphate content of the casein family allows it to associate with calcium and form calcium phosphate salts. In cheese manufacture, casein is cleaved between certain amino acids, and this results in a fragmented protein, which will precipitate.

The behavior of the different types of caseins (α, β, κ) in milk when treated with heat, different pH (acidity), and different salt concentrations provides the characteristics of cheeses. The serum (whey) protein family consists of approximately 50% β-lactoglobulin, 20% α-lactalbumin, blood serum albumin, immunoglobulins, lactoferrin, transferrin, and many minor proteins and enzymes. Like the other major milk components, each whey protein has its own characteristic composition and variations.

Degradation of proteins in cheesemaking occurs essentially by means of enzymes (proteases) coming from several sources: the native milk, airborne bacterial contamination, enzymes or bacteria that are added intentionally, and somatic cells present in milk. Casein proteolysis in cheesemaking starts when rennet (mainly chymosin) is added to milk and the enzyme acts on casein causing the aggregation of casein micelle, which will precipitate (coagulate). Because this enzyme is acidic (its activity is optimized in acidic environment), a starter culture is previously added to milk so to cause a slight, but important, pH lowering. Afterward and during cheese ripening, proteolysis occurs by the action of enzymes native to milk (plasmin) or released by lactic acid bacteria from the starter culture, leading to the formation of smaller fractions such as peptides or even amino acids (Figure 4).

The degree of breakdown of proteins in a cheese can be followed via the analytic method called electrophoresis as shown in Figure 5. The bands in each column represent proteins, polypeptides, or peptides, whose size is more or less inversely proportional to their distance of migration, from top to the bottom of the column. Peptides released during cheese proteolysis have a key role in the development of cheese taste, and a few may also exert specific bioactive or physiological effects such as opioid, antihypertensive, immunomodulatory, and antimicrobial.

Lipids and Lipolysis in Cheese

Fats are made from individual fatty acid molecules attached to glycerol, a three-carbon backbone. The most common type of fat is called a triglyceride, which contains three fatty acids attached to the backbone. Because there are many different fatty acids that can be attached to the backbone (see Figure 6), there are many different types of triglycerides or fats. Fatty acids may be saturated, which means that each carbon has a single bond to another carbon and two hydrogen atoms, or fatty acids may be unsaturated, which means that a carbon has two bonds to the adjacent carbon, called a double bond, and a single bond to another carbon and a hydrogen atom.

Other fatty compounds include phospholipids, which make up approximately 1% of the fat in milk.

Milk contains approximately 3.4% total fat, and cheeses will have an average ten times more fat than milk. Milk fat contains approximately 65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids; thus, cheese is a high-caloric food also containing high levels of saturated fatty acids (butyric, myristic, palmitic, and stearic acids), which,
when consumed in excess may contribute to onset of high blood cholesterol and associated diseases. The conjugated linoleic acid (CLA) is a trans-fatty acid present in milk and cheese that is beneficial to humans in many ways. The biological properties of dietary CLA are attracting considerable interest because studies suggest that it may have a powerful anticarcinogenic, immunomodulating, growth-promoting, lean body mass-enhancing, and antidiabetic properties.

The contents of CLA in cow’s raw milk may vary from 0.2% to 3.7% of total milk fat, and it is established that dairy products derived from ruminants fed predominately on pasture are richer in CLA. Cheeses are thus considered important sources of CLA, with typical content values varying between 8 and 18 mg g⁻¹ of fat.

Lipolysis (degradation of fats by enzymes called lipases) is crucial in the development of cheese aroma and taste. Throughout ripening, lipases transform the milk fat into short-chain fatty acids that may be volatile. The type of the starter culture, the length of the ripening period, and the conditions prevailing are important in defining the rate and type of lipolysis.

The principal carbohydrate in milk is lactose (Figure 7). The concentration of lactose in cow’s milk is relatively constant and averages about 5%.

Glycolysis or fermentation is the chemical reaction by which milk sugar (lactose) is transformed by the microflora into lactic acid and energy and other components.
The accumulation of lactic acid leads to the lowering of the pH of the curd, thus making it inhospitable for many unwanted bacteria and helping in syneresis (whey release). The amount of lactic acid released depends on the starter culture type and on the technological specificities used in the cheesemaking. Long-ripened cheeses may be depleted of lactose making them suitable for consumption by lactose-intolerant consumers.

Cheese Microbiology

Cheesemaking is based on the application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH, thus affecting a
number of aspects of the cheese manufacturing process and ultimately cheese composition and quality.

The LAB or non-LAB microflora of cheese may originate from three main sources: strains present in milk, those of the starter culture added, and those from adventitious ‘in-house’ contamination. While in industrial cheeses, bacteria from the starter culture will dominate from early manufacture to late ripening, traditional cheeses usually owe their typical flavors to the presence of microorganisms from (raw) milk and from ‘in-house’ contamination; in this case, cheeses obtained from distinct milk batches and farms will likely exhibit different numbers of several species. Although several species have been identified in various research works, the groups described in the succeeding text are those most frequently found in traditional cheesemaking.

**Lactic Acid Bacteria**

LAB are the most important group of microorganisms in milk and most dairy products. Species identified in cheese may be homofermentative, such as lactococci, or heterofermentative, such as lactobacilli. Distinct groups of LAB show different degrees of proteolytic activity in cheese, but their main role apparently is the formation of aminic and ammoniacal nitrogen, by degrading peptides and metabolizing amino acids – hence playing a complementary role to rennet in milk protein degradation throughout the whole manufacture process of cheese. The main bacteria associated to cheese and other dairy products are shown in Table 3.

The earliest productions of cheeses were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk and its environment. The quality of the end product was a reflex of the microbial load and spectrum of the raw material. Spontaneous fermentation was later optimized through backslopping, that is, inoculation of the raw material with a small quantity of whey from a previously performed successful fermentation, and the resulting product characteristics depended on the best-adapted strain dominance. Today, backslopping is still used to produce many artisanal raw milk cheeses, that is, inoculation of the raw material with a small quantity of nonstarter lactic acid bacteria from the dairy environment

<table>
<thead>
<tr>
<th>Species/subspecies</th>
<th>Main uses/other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus</td>
<td></td>
</tr>
<tr>
<td>L. lactis subsp. lactis</td>
<td>Mesophilic starter used for many cheese types</td>
</tr>
<tr>
<td>L. lactis subsp. lactis biovar diacetylactis</td>
<td>Used in Gouda, Edam, sour cream, and lactic butter</td>
</tr>
<tr>
<td>L. lactis subsp. cremoris</td>
<td>Mesophilic starter used for many cheese types</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Thermophilic starter used for yogurt and many cheese types particularly hard and semihard high-cook cheeses</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>Probiotic adjunct culture used in cheese and yogurt</td>
</tr>
<tr>
<td>L. delbrueckii subsp. bulgaricus</td>
<td>Thermophilic starter for yogurt and many cheese types, particularly hard and semihard high-cook cheeses</td>
</tr>
<tr>
<td>L. delbrueckii subsp. lactis</td>
<td>Used in fermented milks and high-cook cheese</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>Thermophilic starter for fermented milks and many cheese types particularly hard and semihard high-cook cheeses</td>
</tr>
<tr>
<td>L. casei</td>
<td>Cheese ripening adjunct culture</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>Cheese ripening adjunct culture</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>Cheese ripening adjunct culture</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides subsp. cremoris</td>
<td>Mesophilic culture used for Edam, Gouda, fresh cheese, lactic butter, and sour cream</td>
</tr>
<tr>
<td>Brevibacterium</td>
<td></td>
</tr>
<tr>
<td>B. linens</td>
<td>Used in smear surface-ripened cheeses, Camembert, Stilton, and Limburger and as a cheese-ripening adjunct culture</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td></td>
</tr>
<tr>
<td>P. acidipropionici</td>
<td>Used in Gruyère and Emmental cheeses</td>
</tr>
<tr>
<td>P. freudenreichii subsp. shermanii</td>
<td>Used in Gruyère and Emmental cheeses</td>
</tr>
</tbody>
</table>


Figure 7 Lactose structure made of one molecule of glucose and one molecule of galactose.
Many strains isolated from raw milk cheeses were associated with the formation of more complex volatile profiles and higher scores for some sensory attributes. This, associated with a greater typical microflora biodiversity, makes raw milk cheeses more prone to developing more intense flavor than cheeses made from pasteurized milk.

Molds and yeasts are usually present in raw milk, and they do not survive pasteurization. Thus, unless the cheese is made from raw milk, its contamination with yeasts and molds is essentially caused by reinfection during manufacturing by yeasts and molds present in the environment of cheese factories, like walls and shelves of ripening rooms, air, equipment, water, milk, and brine. Yeasts can metabolize lactic acid in the cheese, causing the rise in pH and leading to the formation of the characteristic undesirable yeasty or fruity flavor and gas in cheeses. However, due the production of metabolites, for example, short-chain fatty acids and other compounds, some specific strains contribute to maturation and aroma formation.

The characteristic feature of some mold-ripened cheese types is extensive proteolysis and lipolysis, releasing peptides and fatty acids that contribute to the development of distinctive flavor and aroma, as in the case of Camembert cheese. Wild types of molds such as Aspergillus flavus may influence the organoleptic characteristics of cheeses or even produce mycotoxins that represent a potential health risk to the consumer. The most common molds found in raw milk cheeses belong to the genera Geotrichum, Aspergillus, Mucor, Fusarium, and Penicillium.

Finally, recall also that poor hygienic practices, inadequate pasteurization, or poor handling during processing may allow for accidental presence of pathogens such as Bacillus cereus, Listeria monocytogenes, Salmonella spp., Escherichia coli O157:H7, Campylobacter jejuni, and Staphylococcus aureus in cheeses.

Figure 8 shows the microflora dynamics throughout ripening of São Jorge raw milk cheese.
Further Reading


Relevant Websites


http://www.thedairysite.com/articles/2875/european-cheese-market — The Dairy Site.

https://www.uoguelph.ca/foodscience/ — University of Guelph.