PART 1

Food from Animal Sources
Secreted from the mammary glands of mammals, milk is a complete food designed to provide newborns with energy, compounds necessary for growth, immunological protection, and so forth, which are all vital in the early stages of life. From a physicochemical point of view, milk is complex in terms of its structure, the interactions between its various components and its variability in composition based on species, breed, diet and lactation period. It is a dynamic system due to the presence of endogenous enzymes and microorganisms as well as ionic equilibria, which depend on pH and temperature, and determine the stability of dispersed elements. These physical, physicochemical and biological changes lead to instability in milk, which can be exploited during processing into a variety of dairy products, such as fermented products, cheese, cream, butter, and so on.

1.1. The biochemistry and physical chemistry of milk

Milk is a natural emulsion. Fat, which represents approximately 4% of the overall composition of cow’s milk (w/w), is present in the form of fat globules dispersed in the skimmed milk phase.

The non-fat phase of cow’s milk (skimmed milk) is composed mainly of water (90% (w/w) of the overall composition) in which the following are dispersed or dissolved:

– lactose (4.8 – 5% (w/w) of overall composition);
– protein (3.2 – 3.5% (w/w));
– non-protein nitrogen (NPN) consisting of urea, amino acids and peptides, representing about 5% of the nitrogen fraction of milk;
– inorganic minerals (calcium, phosphate, chloride, potassium, sodium) and organic acids (mainly citric acid in fresh milk);
– water-soluble vitamins.

1.1.1. Milk fat

The fat content of cow’s milk varies between about 3.3 and 4.7% (w/w) depending on breed, lactation stage, season, and so forth. Milk fat is mostly present in the form of fat globules measuring between 0.2 and 15 µm in diameter. Around 75% of fat globules are smaller than 1 µm, but they represent less than 10% of the total volume of milk fat. Similarly, there are very few fat globules larger than 8 µm; they represent less than 3% of the overall volume. Thus, almost 90% of milk fat is in the form of milk globules measuring between 1 and 8 µm in diameter. The average diameter of fat globules is approximately 4 µm. The core of the fat globule almost exclusively consists of neutral lipids, while the fat globule membrane is composed of complex lipids and proteins. The amphiphilic properties of these complex lipids and proteins facilitate the creation of interfaces and help keep the fat in the dispersed state (Figure 1.1).

**Figure 1.1. Composition and main characteristics of milk fat globules**
1.1.1.1. Composition and characteristics of milk fat

Table 1.1 shows the average lipid composition of cow’s milk. Triacylglycerols represent approximately 97.5% of the total lipids. Diacylglycerols, monoacylglycerols and free fatty acids are naturally present in small amounts but their proportion can increase with lipolysis. The many other compounds (cholesterol, steroid hormones, vitamins, flavorings and flavor substrates, etc.), even though low in number, play a crucial nutritional and sensory role.

<table>
<thead>
<tr>
<th>Class of lipids</th>
<th>Percentage of total lipids (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerols</td>
<td>97.5</td>
</tr>
<tr>
<td>Diacylglycerols</td>
<td>0.36</td>
</tr>
<tr>
<td>Monoacylglycerols</td>
<td>0.027</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.31</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>Traces</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.008</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 1.1. Average lipid composition of cow’s milk (Source: [CHR 95])

Milk triacylglycerols are made up of more than 400 different fatty acids, which makes milk fat a very complex lipid source, as each fatty acid can be esterified to one of the three hydroxyl groups of glycerol (Table 1.2). However, only 12 fatty acids are present in quantities of more than 1% (mol/mol). Fatty acids are either synthesized in the secretory cells in the udder or taken from the bloodstream (body fat or food origin). Thus, milk fat varies depending on the season, the cow’s diet and the energy level of the food intake, which could determine the ratio of de novo synthesis with regard to plasma uptake.
<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Symbol</th>
<th>% mol</th>
<th>Distribution on the glycerol sites (% mol)</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Sn1</strong></td>
<td><strong>Sn2</strong></td>
</tr>
<tr>
<td>Butyric</td>
<td>4:0</td>
<td>4.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caproic</td>
<td>6:0</td>
<td>2.2</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>Caprylic</td>
<td>8:0</td>
<td>1.3</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Capric</td>
<td>10:0</td>
<td>2.9</td>
<td>1.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Lauric</td>
<td>12:0</td>
<td>3.3</td>
<td>4.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Myristic</td>
<td>14:0</td>
<td>10.8</td>
<td>9.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Palmitic</td>
<td>16:0</td>
<td>26.1</td>
<td>34.0</td>
<td>32.3</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1</td>
<td>1.4</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:0</td>
<td>10.8</td>
<td>10.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:1</td>
<td>24.1</td>
<td>30.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:2</td>
<td>2.4</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:3</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1.2. Fatty acid composition of milk and distribution on the three positions of glycerol (adapted from [CHR 95])**

Milk fat is characterized by:

– a high proportion of short-chain fatty acids (chain lengths of four to ten carbons) synthesized from acetate and β-hydroxybutyrate produced by microorganisms during cellulose degradation in the rumen. These fatty acids are preferentially in the Sn3 position of triacylglycerols. They are easily released by the action of microbial or milk lipases, and are actively involved in the flavor of dairy products due to their volatility at acidic pH;

– a high proportion of saturated fatty acids (with 14, 16 and 18 carbon atoms), some of which come from the hydrogenation in the rumen of unsaturated fatty acids originating from food;
– unsaturated fatty acids from either the diet or the desaturation of saturated fatty acids by Δ9-desaturase in epithelial cells;

– unsaturated fatty acids whose double bonds are in trans configuration and/or are conjugated resulting from the hydrogenation of fatty acids in food by microorganisms;

– the presence of bacterial fatty acids (fatty acids with odd numbers of carbons, branched-chain fatty acids).

Fatty acids determine the physical properties of fat (melting point, crystallization properties) by the length of their carbon chain, their level of unsaturation and their position on the glycerol molecule. Milk fat has a broad melting profile that varies throughout the year, mainly due to diet. At -30°C, milk fat is completely solid and at 40°C it is completely liquid. Between these two temperatures, liquid fat, located mainly in the core of the globule, and solid fat, forming a solid shell located at the periphery of the globule, coexist.

1.1.1.2. Milk fat globule membrane

The milk fat globule membrane (MFGM) accounts for 1 – 2% (w/w) of total lipids. It is primarily composed of proteins (butyrophilin, xanthine oxidase, several enzymes, etc.), phospholipids (phosphatidylethanolamine, phosphatidylinositol, phosphatidyl-serine, phosphatidylcholine, and sphingomyelin), neutral lipids (triacylglycerol) and a small proportion of other components (cholesterol, cerebrosides, β-carotene, etc.). Its structure is closely linked to the mechanisms involved in the formation of lipid droplets in secretory cells and to their method of secretion in the alveolus of the mammary gland. It is composed of an inner layer of proteins and polar lipids from the endoplasmic reticulum, allowing the lipid fraction to be dispersed as lipid droplets in the cytoplasm of secretory cells. These lipid droplets, when secreted, are surrounded by the phospholipid bilayer membrane of secretory cells (Figure 1.2). A portion of the cytoplasm from the secretory cells can be trapped in the MFGM. The membrane is typically around 40 nm thick. The MFGM is composed of lipid rafts, consisting of rigid domains rich in sphingomyelin, which move in a continuous bilayer made of the other phospholipids. The size of the lipid rafts depends on the temperature and time of milk fat globule handling.
Due to the composition of the MFGM, the interfacial tension between the fat phase and skimmed milk is low at around 2 mN m\(^{-1}\), which makes it very sensitive to local perturbations. The total surface of the MFGM is around 80 m\(^2\) L\(^{-1}\) of fresh milk. This can, however, be considerably increased during processing (agitation, homogenization, etc.). The surface electrostatic potential of the fat globule, which is close to \(-13\) mV at the natural pH of milk, contributes to stability by limiting the risk of flocculation and coalescence.

### 1.1.2. Carbohydrates

Milk contains free carbohydrates, the main one being lactose, and carbohydrates bound to proteins. The lactose concentration in mammalian milk is inversely proportional to the mineral content, both of which contribute to the balance of osmotic pressure. The lactose content of cow’s
milk varies from 4.8 to 5% (w/w) and represents 97% of total carbohydrates. Lactose is a disaccharide composed of a galactose and a glucose unit (Figure 1.3). It is made from blood glucose in the presence of galactosyltransferase and α-lactalbumin. For absorption, lactose should be hydrolyzed by β-galactosidase (lactase) secreted by enterocytes in the small intestine. The low hydrolysis rate of lactose provides young mammals with prolonged energy and a constant blood glucose level between feedings. Lactase-deficient individuals cannot digest lactose as it provokes intestinal problems (diarrhoea, bloating) when ingested. Galactose and its amino derivative galactosamine contribute to the formation of several glycoproteins and/or glycolipids.

![Figure 1.3. Chemical structure of lactose](image)

Lactose has a low solubility (around 18 g/100 g of water at 20°C) compared to other carbohydrates: it can crystallize when concentrated in the aqueous phase of milk or derivatives (evaporation, freezing, storage in powder form). Lactose has a high melting point for a disaccharide (over 200°C). It has a low sweetness level (0.3 with reference to sucrose, which has a sweetness level of 1). Lactose has one reducing function per molecule, carried by the glucose unit. It is thus prone to non-enzymatic browning, which changes the flavor and color of foods (Maillard reaction). Enzymatic hydrolysis by β-galactosidase combats lactose intolerance, improves the sweetness of milk and doubles its reducing power, which promotes non-enzymatic browning. Lactose is the main substrate for lactic acid bacteria. The transformation of lactose to lactic acid lowers the pH of milk and destabilizes the dispersed elements, which is the basis of the production of fermented dairy products.
1.1.3. Proteins

Cow’s milk contains 3.2 – 3.5% (w/w) protein, which can be divided into two separate fractions:

– caseins that precipitate at pH 4.6, representing 80% of total protein;
– whey proteins, soluble at pH 4.6, representing 20% of total protein.

Differential protein precipitation is used industrially in the preparation of acid casein. Casein exists as micelles comprising colloidal minerals mostly in the form of calcium phosphate as described later.

1.1.3.1. Caseins

Caseins (αS1, αS2, β, κ), present in cow’s milk in proportions of 37, 10, 35 and 12%, respectively (w/w), are synthesized from four different genes. Protein diversity is increased by the presence of numerous variants resulting from genetic polymorphism and differences in post-translational modifications (phosphorylation, glycosylation).

Caseins are small proteins with a molecular weight of 19 – 25 kDa. They have a high proportion of non-uniformly distributed charged amino acids and non-polar amino acids (Table 1.3), giving them amphipilic properties.

Due to the presence of a large number of proline residues, caseins have a low level of secondary structure (α-helices or β-sheets). Thus, caseins can withstand intense heat treatment but are very sensitive to enzymatic action, in particular digestive enzymes (pepsin and trypsin). β-casein is particularly sensitive to plasmin, an endogenous milk protease found on the surface of casein micelles. The hydrolysis of β-casein by plasmin generates hydrophilic peptides from the N-terminal fragments of β-casein and hydrophobic γ-caseins, which precipitates at pH 4.6, similar to other caseins.

Caseins are rich in lysine, an essential amino acid that in the presence of a reducing sugar is heavily involved in non-enzymatic browning. However, the large number of acidic amino acids gives caseins an isoelectric point of close to 4.6 at the ionic strength of milk.
Table 1.3. Amino acid composition of cow’s milk casein (α_{S1} variant B), α_{S2} variant A, β variant A, casein κ

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>α_{S1} (B)</th>
<th>α_{S2} (A)</th>
<th>β (A)</th>
<th>κ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Glu</td>
<td>24</td>
<td>25</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Asn</td>
<td>8</td>
<td>14</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Gln</td>
<td>15</td>
<td>15</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Thr</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Ser</td>
<td>8</td>
<td>6</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>SerP</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Pro</td>
<td>17</td>
<td>10</td>
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<td>20</td>
</tr>
<tr>
<td>Gly</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Ala</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Val</td>
<td>11</td>
<td>14</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Ile</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Leu</td>
<td>17</td>
<td>13</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Phe</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Tyr</td>
<td>10</td>
<td>12</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Met</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Cys</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 or 2</td>
</tr>
<tr>
<td>Cystine/2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2 or 0</td>
</tr>
<tr>
<td>Lys</td>
<td>14</td>
<td>24</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>His</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Arg</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Trp</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>207</td>
<td>209</td>
<td>169</td>
</tr>
</tbody>
</table>

The isoelectric point of casein is closely linked to the phosphoserine content. Caseins, with the exception of κ-casein, contain a high proportion of phosphorylated serine predominantly arranged in clusters (sequence of phosphoserines in the primary structure). α_{S1}-casein mostly has eight phosphoserines. α_{S2}-casein mainly has ten to 13 phosphoserines in almost equivalent proportions. β-casein contains five phosphoserines whereas κ-casein mainly has one. However, κ-casein is the only protein that can
sometimes be glycosylated. Phosphoserines, arranged in clusters, display a strong affinity for divalent or polyvalent cations, which depending on their type can make casein insoluble. The sensitivity of casein to calcium increases with the rate of phosphorylation. κ-casein does not precipitate in the presence of calcium.

Caseins have few sulfuric amino acids, which limits their nutritional value. \( \alpha_{s2} \)- and κ-caseins each have two cysteines involved in intermolecular disulphide bonds. While \( \alpha_{s2} \)-casein is mostly present as covalent homodimers, κ-casein forms polymers of up to 15 κ-casein units.

### 1.1.3.2. Structure of the casein micelle

Casein micelles are spherical particles formed by the aggregation of different caseins (\( \alpha_{S1}, \alpha_{S2}, \beta \) and \( \kappa \)), some peptide fragments resulting from the proteolysis of \( \beta \)-casein by plasmin (\( \gamma \)-casein) and salt components, the main ones being calcium and phosphate. Table 1.4 shows the average composition of casein micelles.

<table>
<thead>
<tr>
<th>Caseins</th>
<th>Salt components</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_{S1} )</td>
<td>33</td>
<td>Calcium</td>
</tr>
<tr>
<td>( \alpha_{S2} )</td>
<td>11</td>
<td>Magnesium</td>
</tr>
<tr>
<td>( \beta )</td>
<td>33</td>
<td>Inorganic</td>
</tr>
<tr>
<td>( \kappa )</td>
<td>11</td>
<td>Citrate</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total caseins</td>
<td>92</td>
<td>Total salt components</td>
</tr>
</tbody>
</table>

**Table 1.4. Average composition of casein micelles in % (w/w)**

The composition of casein micelles varies slightly depending on their diameter, which varies between 50 and 600 nm for an average diameter of about 150 nm. Regardless of the size of the micelle, the proportion of \( \alpha_{S1} \) and \( \alpha_{S2} \) casein varies marginally, whereas the ratio of \( \beta \) to κ-caseins increases with the size of the micelle. Micelle organization, or the arrangement and distribution of the various micelle components and their types of association, is still a subject of intense debate. The non-charged regions of caseins form rigid structures maintained by hydrophobic associations and hydrogen bonds; colloidal calcium phosphate, in the form of nanoclusters, shields the negative
charges of phosphoserine clusters and allows the association of casein micelles. κ-casein is structured into inhomogeneous clusters almost exclusively located on the micelle surface. Without phosphoserine clusters, κ-casein remains associated to the casein micelle by its hydrophobic N-terminus, but prevents further micelle growth. Its charged hydrophilic C-terminus protrudes about 5 – 10 nm into the solvent phase, making the micelle appear hairy.

Table 1.5 shows some of the properties of casein micelles. Their composition and physicochemical properties are highly dependent on the solvent phase.

<table>
<thead>
<tr>
<th>Property</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average diameter (nm)</td>
<td>150</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>$8 \times 10^{-10}$</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>$2.1 \times 10^{-15}$</td>
</tr>
<tr>
<td>Density (hydrated)</td>
<td>1.0632</td>
</tr>
<tr>
<td>Hydration (g H₂O g⁻¹ of proteins)</td>
<td>3.7</td>
</tr>
<tr>
<td>Voluminosity (mL g⁻¹ of proteins)</td>
<td>4.4</td>
</tr>
<tr>
<td>Molecular weight (hydrated) (Da)</td>
<td>$1.3 \times 10^9$</td>
</tr>
<tr>
<td>Molecular weight (dehydrated) (Da)</td>
<td>$5 \times 10^8$</td>
</tr>
<tr>
<td>Water content (%, w/w)</td>
<td>63</td>
</tr>
<tr>
<td>Number of caseins per micelle</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td>Number of nanoclusters of calcium phosphate per micelle</td>
<td>$3 \times 10^3$</td>
</tr>
<tr>
<td>Number of micelles per L of milk</td>
<td>$10^{17} – 10^{19}$</td>
</tr>
<tr>
<td>Mean free distance between micelles (nm)</td>
<td>240</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>−13</td>
</tr>
</tbody>
</table>

**Table 1.5. Average physicochemical properties of casein micelles at 20°C and pH 6.7 (modified from [MCM 84])**

### 1.1.3.3. Whey proteins

Whey proteins are defined as the protein fraction that remains soluble at pH 4.6. β-lactoglobulin, α-lactalbumin, bovine serum albumin (BSA), immunoglobulins and lactoferrin represent more than 90% of all whey proteins. They are mostly globular proteins with a high sensitivity to heat
treatment. They are generally rich in sulfuric amino acids and tryptophan residues making them highly nutritious.

β-lactoglobulin has a molecular weight of 18.3 kDa and its concentration in cow’s milk ranges from 0.2 to 0.4% (w/w). Its biological function is still unknown. There are several genetic variants of β-lactoglobulin, but types A and B are the most common. Its secondary structure consists primarily of two perpendicular β-sheets forming a central hydrophobic cavity held in place by two disulphide bridges and partially closed by an α-helix. The cavity can hold a small hydrophobic molecule, which can be a fatty acid, retinol or an aromatic molecule. In addition, β-lactoglobulin has a free cysteine residue naturally buried in the protein core, which upon input of energy (e.g. heat) is exposed to the solvent and can initiate intermolecular exchange reactions. β-lactoglobulin has a pI of 5.2 and its quaternary structure varies depending on pH. Under physiological conditions (pH 6.8), β-lactoglobulin exists mainly in the form of non-covalent dimers.

α-lactalbumin has a molecular weight of 14.1 kDa and a pI of 4.5. Its concentration in cow’s milk ranges from 0.1 to 0.15% (w/w). The secondary structure of α-lactalbumin consists of four α-helices and a β-sheet; its tertiary structure is stabilized by four disulphide bridges and the presence of one calcium ion at a specific site on the protein. The affinity of α-lactalbumin for calcium and its conformation are highly dependent on pH. A drop in pH below 4 induces protonation of carboxylic groups involved in the coordination of calcium, which results in the release of calcium. α-lactalbumin contributes to the regulation of galactosyltransferase activity in the synthesis of lactose.

BSA is present in cow’s milk at a concentration of between 0.01 and 0.04% (w/w). Its molecular weight is 66 kDa and it has the distinction of having 35 cysteine residues, 34 of which are involved in intramolecular disulphide bridges. It has an ellipsoidal shape and its surface is comprised of hydrophobic pockets allowing the attachment of long-chain fatty acids.

Immunoglobulins are present in cow’s milk at a concentration of 0.06 – 0.1% (w/w). Their pI is within a pH range of 5 – 8. They are glycoproteins derived from blood and have antibody properties. They are synthesized in response to stimulation by antigens. Immunoglobulins are comprised of two types of polypeptide chains, a light chain with a molecular weight of about 28 kDa and a heavy chain of about 50 – 70 kDa. The basic structure of
immunoglobulins, the molecular weight of which is close to 160 kDa, consists of four subunits linked by disulphide bonds. Each subunit differs in its amino acid sequence at the N-terminus, which gives the subunits immunological specificity.

Lactoferrin has a molecular weight of 75 kDa and a pI of 8.5. Its tertiary structure is stabilized by 16 disulphide bridges. It has two free cysteine residues. A molecule of lactoferrin has the ability to bind two iron ions in the presence of a synergistic anion (carbonate under physiological conditions). Affinity to iron is high at neutral or basic pH (stabilization of iron in basic medium), but iron is quickly released in acid medium. Its iron chelating property gives lactoferrin antimicrobial activity.

1.1.4. Milk minerals

Although the mineral fraction of milk is relatively small, it is very important from a structural, nutritional and technological point of view. Calcium phosphate nanoclusters, associated with casein phosphoserins $\alpha_{S1}$, $\alpha_{S2}$ and $\beta$, contribute to the structure and stability of casein micelles (see section 1.1.3.2). The solubilization of colloidal calcium phosphate in the presence of a calcium complexing agent such as EDTA (ethylene diamine tetra-acteic acid) results in disintegration of the micelle. Milk and milk derivatives are the main supply of calcium and phosphorus in the diet. In cheese-making, the rheological properties of cheese strongly depend on the retention of these elements in the curd. Table 1.6 shows the average concentrations of the main minerals in cow’s milk.

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Concentration (mg kg$^{-1}$)</th>
<th>Concentration (mmol kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>1,043–1,283</td>
<td>26–32</td>
</tr>
<tr>
<td>Magnesium</td>
<td>97–146</td>
<td>4–6</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>1,805–2,185</td>
<td>19–23</td>
</tr>
<tr>
<td>– (total phosphorus)</td>
<td>930–992</td>
<td>30–32</td>
</tr>
<tr>
<td>Citrate</td>
<td>1,323–2,079</td>
<td>7–11</td>
</tr>
<tr>
<td>Sodium</td>
<td>391–644</td>
<td>17–28</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,212–1,681</td>
<td>31–43</td>
</tr>
<tr>
<td>Chloride</td>
<td>772–1,207</td>
<td>22–34</td>
</tr>
</tbody>
</table>

Table 1.6. Mineral composition of cow’s milk (according to [GAU 05])
Milk also contains many trace elements. The concentration of mineral elements is not influenced by diet, even though differences are observed for citrate. However, there are more variations during lactation or with pathological conditions (mastitis).

Mineral elements are distributed differently between the soluble phase and the colloidal phase, depending on their respective affinities for proteins and organic solutes. Monovalent ions (sodium, chloride, potassium) are found exclusively in the soluble phase of milk, while divalent or polyvalent ions are distributed between both phases. The mineral balance between the colloidal phase and the soluble phase is rather complex, since many different types of minerals are involved. Figure 1.4 shows the main mineral balances of milk.

![Figure 1.4. Main mineral balances of milk (salt concentrations shown are for milk under physiological conditions)](image)

Calcium phosphate is poorly soluble and is saturated in the soluble phase of milk (0.59 mM) over a wide pH range. In milk at pH 6.7, the natural content of calcium phosphate is far greater than its solubility limit. Micelles increase the solubility of calcium phosphate by the integration of calcium phosphate nanoclusters with a core-shell structure: calcium phosphate clusters stabilized by the phosphorylated caseins $\alpha_S1$, $\alpha_S2$ and $\beta$. In addition, a fraction of calcium is directly linked to casein phosphoserines. Under physiological conditions, approximately two-thirds of calcium and half of the inorganic phosphate are associated with micelles and are in equilibrium with the serum phase. Any physicochemical change in milk will affect the concentration of minerals in the soluble phase of milk, causing a shift in the mineral balance and an alteration in the structure and stability of micelles (see section 1.2.2).
1.2. Biological and physicochemical aspects of milk processing

1.2.1. The stability of fat globules

1.2.1.1. Native fat globules

Native fat globules have a natural tendency to rise to the surface (creaming), the rate of which depends on globule size, the temperature affecting the viscosity of the continuous phase, the difference in density between the continuous and dispersed phases and the gravitational acceleration (Stokes’ law, equation [1.1], Volume 2). These characteristics are taken into account in the preparation of milk and cream for consumption. Flocculation and the coalescence of fat globules accelerate creaming by increasing particle size. In milk, flocculation or agglutination of fat globules is mainly due to the IgM class of immunoglobulins that result in the formation of aggregates of up to 1mm (up to $10^6$ fat globules). These aggregates form at cold temperatures and can be separated by stirring or reheating milk above 37°C. Fat globules of heat-treated milk are less prone to agglutination due to IgM denaturation. Coalescence of fat globules does not occur in milk emulsions due to the electrostatic barrier generated by the charge of the native MFGM (surface potential of $-13$mV at the natural pH of milk) and the steric barrier formed by hydrophilic carbohydrate chains of glycoproteins in the membrane. Lowering pH that reduces the MFGM surface potential or increasing the ionic strength that reduces the thickness of the electrical double layer, decreases emulsion stability by promoting flocculation and the coalescence of fat globules. In addition, coalescence of fat globules can be obtained by the vigorous stirring of cream (churning) or by a series of freeze–thaw cycles, which have a destabilizing effect on the membrane.

Partial coalescence occurs when fat globules, the fat of which is partially crystallized, aggregate but keep their shape after contact despite perforation of their membrane by lipid crystals. The mechanical rigidity provided by fat crystals on the surface of the fat globules prevents complete fusion. Partial coalescence is favored by the low interfacial tension and low viscoelasticity of the native MFGM.

Fats are also susceptible to biological (lipolysis) or chemical (oxidation) degradation. In fresh cow’s milk, lipolysis and oxidation of fat are virtually non-existent despite the natural presence of lipoprotein lipase (lipolysis catalyst), oxygen and oxidation catalysts dissolved in the non-fat phase. The native MFGM, although relatively weak due to low interfacial tension (around
2 mN m\(^{-1}\)) and the large radius of curvature, forms a protective layer against such reactions. However, any change to the native MFGM increases the risk of lipolysis and oxidation of milk fat.

### 1.2.1.2. Homogenized fat globules

Changing the physicochemical characteristics of an emulsion affects its properties. Homogenization, for example, improves the physical stability of emulsions by reducing the average diameter of the fat globules, and thus lowers the rate of creaming. The membrane becomes thicker and more viscoelastic due to the adsorption of casein micelles and whey proteins to the newly-formed interface (Figure 1.5); this limits the possibility of penetration of fat crystals and therefore reduces the risk of partial coalescence of the fat globules. However, the strong increase in the interfacial area and the change in the nature of the membrane due to homogenization alter its protective properties against oxidation and lipolysis.

![Figure 1.5. Structure of the milk fat globule membrane after homogenization. For a color version of this figure, see www.iste.co.uk/jeantet/foodscience.zip](image)

In addition, homogenization changes the color of milk emulsions as well as the participation of fat globules in the formation of coagulum (cheese, yoghurt). Native fat globules cannot participate in the formation of the protein network. Moreover, if the diameter of the fat globules is greater than 1 µm, they even hinder the network formation. On the other hand, homogenized fat globules are involved in the formation of the protein network via the casein micelles incorporated into the interface created during homogenization. In low-fat products, homogenization (one stage) can be a means to increase the viscosity of dairy emulsions; in such systems, linear aggregates of flocculated fat globules are formed. Homogenization also causes an increase in the interfacial tension between the lipid and aqueous phases, which, together with
a reduction in fat globule size makes the interface more resistant to mechanical processing and phase inversion. Greater stability of fat globules resulting from changes to the interface may have an adverse effect on the rheological, sensory and culinary properties of cheese (altered melting properties after homogenization).

1.2.2. Protein stability

Casein micelles and whey proteins differ in their resistance and technological stability during the processing of milk. Maintaining the micellar structure primarily depends on colloidal calcium phosphate, which acts within the micelles. On the other hand, the C-terminal part of \( \kappa \)-casein forms highly hydrated and negatively-charged protrusions on the micelle structure, which hinder casein micelle self-association. Many technological processes adversely affect stability by modifying the physicochemical properties of the micelle surface. Figure 1.6 shows the impact of the main physicochemical factors on the structure and stability of casein micelles.

![Figure 1.6. Impact of the main physicochemical factors on casein micelle structure and stability](image)

The stability of whey proteins is governed by a set of low-energy bonds (hydrogen bonds, hydrophobic interactions, electrostatic bonds, salt bridges) and covalent bonds (disulphide bridges).
1.2.2.1. **Effect of temperature**

Temperature affects the solubility of calcium phosphate (inverse solubility salt) as well as the state of association of milk proteins. Both refrigeration and heat treatment alter the technological properties of the casein micelle, but the underlying mechanisms are different.

Partial solubilization of colloidal calcium phosphate (about 10%) during the cooling of milk (4°C) is reversible upon heating. In addition, the disassociation of β-casein from the micellar structure occurs at low temperature due to a reduction in hydrophobic interactions; once in the soluble phase, it can be hydrolyzed by plasmin (endogenous milk enzyme), which results in a decrease in cheese yield (see section 1.3.4). β-casein and/or hydrophobic fragments resulting from its hydrolysis by plasmin associate with the micelles during the heating of refrigerated milk. As a consequence, the rennet coagulation of such milk is altered. Indeed, it is likely that β-casein, mostly located in the center of the native micelle, moves on its surface during the refrigeration–heating cycle of milk. Its presence on the micelle surface could reduce chymosin site accessibility on κ-casein.

Unlike whey proteins, casein micelles are relatively stable to heat treatment. Heat treatment decreases the solubility of calcium phosphate, which either insolubilizes inside the casein micelle or precipitates on the exchanger surface. The latter fraction is not recovered during the cooling of milk. If the heat treatment is below 95°C for a few seconds, the calcium phosphate insolubilized inside the micelle remains in equilibrium with the soluble phase of milk and resolubilizes during cooling. For more intense heat treatment (e.g. 120°C for 20 minutes), irreversible changes take place in the structure and distribution of salts between the micelle and the soluble fraction. At temperatures above 70°C, whey proteins denature and can interact with each other in the soluble phase of milk (formation of soluble aggregates) or with κ-casein (formation of stable aggregates on the micelle surface). The distribution of aggregates between the soluble phase or the micelle surface depends on pH and determines the heat stability of milk. The heat treatment of milk with a pH above 6.7 promotes the release of κ-casein, which decreases micelle stability. When heat treatment is carried out at a pH below 6.6, a large proportion of whey proteins remain associated with the casein micelle. Thus, the stability of heat-treated milk is greatest when the heat treatment is carried out between pH 6.6 and 6.7. Aggregation of whey proteins on the casein micelle surface makes them stable to chymosin hydrolysis by masking the cleavage site on κ-casein.
In addition, heat treatment applied to milk (e.g. 95°C for a few minutes) has a positive effect on the texture of the gels obtained after slow acidification (yoghurt).

On another level, the interaction of lactose with proteins during heat treatment (Maillard reaction) may alter their functional characteristics.

1.2.2.2. Effect of concentration

The concentration of milk by evaporation increases the colloidal calcium phosphate content of casein micelles. It also increases ionic strength and decreases the pH of milk, resulting in the shielding of the negative charges on the C-terminal portion of κ-casein. In contrast, the concentration of milk by ultrafiltration does not alter the mineral concentration of the soluble phase and therefore does not affect the structure and stability of casein micelles.

1.2.2.3. Effect of ionic environment

Calcium (generally calcium chloride) is widely used in cheese technology to offset the adverse effects of heat treatment and to improve the rheological properties of curd. It induces major changes in the distribution of salts between the soluble and the colloidal phase. It leads to the formation of calcium phosphate (CaHPO₄), which, given its low solubility, mainly insolubilizes inside casein micelles. In addition, some of the calcium ions reduce the zeta potential of the micelle and its thermal stability. At the same time, they cause a decrease in the level of hydration in the micelle.

The addition of sodium chloride causes an increase in ionic strength and a decrease in the activity coefficient of ions in the soluble phase. This results in the solubilization of colloidal calcium phosphate. Hydration of the casein micelles increases without any change in its size and its surface potential.

Citrate is a commonly used complexing agent of calcium. Its addition to milk causes a shift in equilibrium, which results in a dissociation of calcium phosphate and the solubilization of colloidal calcium phosphate. Depending on the amount added, citrate can cause the disintegration of the casein micelles and the release of free caseins. Unlike citrate, phosphate addition increases the calcium phosphate content of the casein micelles. By reducing the amount of ionic calcium, phosphate and citrate increases the thermal stability of milk.
1.2.2.4. Effect of acidification

The acidification of milk causes major physicochemical changes to both the casein micelle and serum. Rapid acidification of milk (concentrated organic or inorganic acid) causes destabilization of the casein micelle surface and flocculation of casein micelles in the form of a precipitate of varying granular size dispersed in whey. Slow acidification (lactic acid bacteria, glucono-delta-lactone) causes a greater rearrangement of casein micelles leading to the formation of a homogeneous gel throughout the entire milk volume. During slow acidification (Figure 1.7), the surface potential of casein micelles decreases gradually. At the same time, the protonation of citrate and phosphate causes the dissociation of soluble calcium salts (mainly calcium phosphate and calcium citrate) and a shift in the mineral balance of milk resulting in the solubilization of colloidal calcium phosphate and in the release of some caseins from the casein micelle. Up to a pH of 5.4, the solubilization of colloidal calcium phosphate has little impact on the organization of the micelle. At a pH below 5.4, the release of calcium bound to phosphoserines causes a gradual disintegration of the micelle, which loses its spherical shape. In addition, the amount of soluble caseins (mostly β casein) reaches a maximum between pH 5.5 and 5.2 (10 – 30%, depending on temperature). When the surface charge of the micelles is zero (pH 5.2), their distribution, homogenous until then, becomes inhomogeneous. The disintegrated casein micelles form aggregates of a few µm dispersed in the whey, which are progressively connected by the solubilized caseins. This results in the formation of a gel network containing the entire aqueous phase, which contracts continuously when the pH decreases from pH 5.0 to approximately 4.4 [HEE 85].

1.2.2.5. Effect of renneting

Rennet, a mixture of chymosin and pepsin, is the coagulating enzyme of casein and is widely used in cheese technology. The destabilization of the casein micelle by rennet resulting in the formation of a gel can be divided into three stages (Figure 1.8):

– enzymatic hydrolysis of κ-casein;
– aggregation of hydrolyzed casein micelles;
– reorganization of the aggregated casein micelles and formation of a gel network.
Hydrolysis of the Phe$_{105}$–Met$_{106}$ bond in κ-casein is accompanied by the release of the hydrophilic and negatively-charged C-terminal segment (caseinomacropeptide CMP) in whey, whereas the hydrophobic and basic N-terminal remains associated with the micelle. The release of CMP destabilizes the colloidal complex by reducing the surface potential of casein micelles. The rate of hydrolysis is considered to be Michaelis–Menten type.
kinetics (see Volume 2, Chapter 7) in which the Michaelis constant ($K_m$) is much greater than the concentration of $\kappa$ casein ($\kappa$):

$$\frac{-d\kappa}{dt} = \frac{k_\theta E_t \kappa}{K_m + \kappa}$$

[1.1]

with $\frac{-d\kappa}{dt}$ being the rate of hydrolysis of $\kappa$ casein, $k_\theta$ (s$^{-1}$) the rate constant of the enzymatic reaction and $E_t$ the total enzyme concentration.

When the repulsive forces (electrostatic and steric) responsible for colloidal stability are neutralized, which is achieved at 80% hydrolyzed $\kappa$-casein, close or adjacent casein micelles aggregate (second-order kinetics):

$$\frac{dn}{dt} = -k_a n^2$$

[1.2]

where $n$ denotes the number of casein micelles at time $t$, and $k_a$ the aggregation constant.

The aggregation of destabilized casein micelles is driven by electrostatic interactions between oppositely-charged residues, hydrophobic bonds and presumably calcium bridges. The rate of aggregation quickly increases with the increasing rate of hydrolysis of $\kappa$-casein between 80 and 100% through the rapid increase in the aggregation constant ($k_a$):

$$k_a = k_{a0} e^{-\frac{\psi (1 - \chi)}{kT}}$$

[1.3]

where $k_{a0}$ is the aggregation rate constant of uncharged particles (mol$^{-1}$ s$^{-1}$), $\psi$ is the repulsion potential energy between casein micelles (J), $\chi$ is the rate of hydrolysis (%), $k$ is the Boltzman constant (J K$^{-1}$) and $T$ is temperature (K).

During aggregation, the equilibration of soluble calcium with colloidal calcium phosphate leads to a major reorganization of casein micelles and the formation of a gel. The rate of change of the rheological properties of the gel is highly dependent on the concentration and the availability of calcium.
1.3. Dairy product technology

The processing of milk into dairy products is based on the influence of biochemical (composition), physicochemical (pH, ionic strength, intensity [time/temperature] of the heat treatment) and biological factors (enzyme or flora action) on the stability of milk. A distinction can be made between:

- products for which a high level of biological and physicochemical stability is desired (liquid milk, milk powder);
- products, such as fermented milks, which associate the physicochemical destabilization of the milk during acid coagulation and its biological stability up to consumption (risk associated with the presence of pathogens, exudative phenomena and post-acidification);
- products resulting from the separation and concentration of all or part of the more valuable fractions of milk (protein and/or fat) by exploiting their instability (butter, cheese).

1.3.1. Liquid milk

The changes in technological processing, preservation techniques and distribution have allowed the development of a wide range of liquid milk (i.e. drinking milk) that differs in its composition, nutritional and sensory quality and shelf-life. Global market trends show a strong decrease in the consumption of whole milk (3.6% fat (w/w)) in favor of semi-skimmed milk (1.5 – 1.8% fat (w/w)), skimmed milk (less than 0.3% fat (w/w)) and “special” milks (infant formula milk, milk fortified with vitamins, calcium, phosphorus, magnesium and/or fiber, organic milk, growth milk, flavored milk, lactose-free milk, etc.).

Milk for human consumption can be currently classified into three categories:
- untreated raw milk;
- heat-treated milk;
- microfiltered milk.

These milks are only subject to physical treatment such as fat and/or protein, mineral and vitamin standardization, homogenization to avoid creaming, and heating or cross-flow microfiltration to reduce microorganisms.
1.3.1.1. Raw milk

The production and sale of raw milk must be highly controlled due to potential health risks. Milk should come from:

– registered healthy animals free from brucellosis and tuberculosis;
– registered farms, subject to strict veterinary control;
– a process (milking, packaging, storage) that is carried out under good hygienic conditions.

Authorities specify the conditions of production and the microbiological quality standards of raw milk.

1.3.1.2. Heat-treated milk

Depending on the intensity of the heat treatment (see Chapter 4, Volume 2), a distinction can be made between:

– pasteurized milk;
– long-life milk.

Pasteurized milk

Pasteurization is used to destroy all pathogenic microorganisms in milk (Figure 1.9). The destruction of tubercle bacillus is often taken as a reference for the choice of pasteurization level. Pasteurization levels are defined by equivalent temperature/time relationships based on a $z$ value of 5°C: the time is reduced by a factor of 10 for a temperature increase of 5°C.

![Figure 1.9. Time–temperature diagram of pasteurization](image-url)
Two types of heat treatment are generally used for milk:

- **High temperature short time (HTST) pasteurization** (71–72°C/15–40 s): This is used for high-quality raw milk. From a sensory and nutritional perspective, high pasteurization has little impact: alkaline phosphatase is inhibited but peroxidase remains active. The use-by date of HTST pasteurized milk is 7 days after packaging (glass bottle, carton, polyethylene or aluminium container).

- **Flash pasteurization** (85 – 90°C/1 – 2 s): This is used for poor quality raw milk. Phosphatase and peroxidase are inhibited.

**Long-life milk**

Long-life milk has undergone sterilization, the purpose of which is to destroy all microorganisms; in return, the sensory and nutritional quality is altered compared to pasteurized milk. Sterilization levels are defined based on a 12 decimal reduction of *Clostridium botulinum*. In long-life milk, shelf-life is limited by slow time-dependent physicochemical changes in the product (precipitation, gelation, etc.).

**Sterilized milk**

Milk is pre-sterilized (135–150°C/3–10 s) after homogenization (in the case of milk containing fat). It is then cooled to 70–80°C and bottled (high-density polyethylene) before undergoing a second sterilization (115°C/15–20 min) followed by rapid cooling. This has a negative impact on color and flavor due to the Maillard reaction. The shelf life is around 150 days. In order to prevent lipid oxidation, such milk is stored away from light, generally in opaque containers. From a nutritional perspective, such heat treatments lead to a loss of thiamine and vitamins B₁₂ and B₆.

**Ultra high temperature (UHT) milk**

Milk is heated to 135–150°C for 1–6 s. This process helps to preserve the original nutritional and sensory qualities of the milk because the z value of the Maillard reaction is greater than that of microbial inactivation. Its shelf life is around 120 days. This limit is imposed to ensure physicochemical stability against precipitation, flocculation and gelation due to the partial proteolysis of casein by residual plasmin or heat-resistant bacterial proteases.
UHT treatment is either direct or indirect, depending on the materials used:

– in the case of direct UHT treatment, food-grade steam is injected into milk preheated to 80°C, where it condenses releasing the latent heat of evaporation. The resulting dilution is corrected during cooling by expansion of the mixture in a partial vacuum chamber;

– in the case of indirect treatment, there is no contact between the milk and the steam. The treatment is carried out with plate or tubular heat exchangers. The limiting factor of the process is the gradual fouling caused by the precipitation of protein/mineral complexes on the walls of the exchanger:

- homogenization is carried out in either the rising or the falling phase; in the latter case, it is necessary to ensure sterilization of the homogenizer,

- the intensity of heat treatments applied is related to the quantity of lactulose in UHT milk.

Figure 1.10. Processing diagram for the production of microfiltered whole milk (VRF = Volume reduction factor)

1.3.1.3. Microfiltered milk

Microfiltration (1.4 µm) is used to obtain liquid milk with the original flavor intact and a shelf life of around 21 days. Dispersed elements and microorganisms are concentrated at temperatures of around 50°C in the retentate (often called “bacterial retentate”), while all other constituents are transferred to the permeate (microfiltrate; Figure 1.10). In order to increase
yield and the number of decimal reductions obtained, double filtration is generally performed to achieve a volume reduction factor (VRF) of 200. Milk is pre-skimmed and the cream is reincorporated to the microfiltrate after a specific heat treatment.

Combined with a moderate heat treatment, the shelf life of microfiltered milk can range from 35 days (treatment of 20 s at 72°C) to six months (treatment of 6 s at 96°C).

1.3.2. Fermented milk products

The bacterial conversion of lactose forms the basis of a wide variety of fermented products (yoghurt, kefir, kumis, etc.) and is one of the oldest methods used for stabilizing milk. Fermentation causes the formation of an acidic (or alcoholic) gel consisting of a network of proteins and fat globules trapped in the aqueous phase. Yoghurt is the most popular fermented milk product and is obtained exclusively by the growth of lactic acid bacteria Streptococcus salivarius subsp thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, which should be inoculated simultaneously. Any products containing bacteria other than these cannot be called yoghurts but are fermented milk. In yoghurt, lactic acid bacteria should be viable, active and present in abundant quantities (∼10⁷ bacteria g⁻¹); the lactic acid content must not be less than 0.7 % (w/w) in products sold to consumers [MAH 00]. Many molecules generated during fermentation, other than lactic acid, contribute to the sensory (diacetyl, acetaldehyde, etc.) and health (bioactive peptides, β-galactosidase, etc.) qualities of fermented products.

There are two types of yoghurts: set and stirred yoghurt. In the case of set yoghurt, fermentation occurs directly in the container; these are usually natural or flavored yoghurts. In the case of stirred yoghurt, fermentation occurs in tanks prior to stirring, smoothing (up to total liquefaction of the gel in the case of drinking yoghurts) and packaging; these are generally smooth natural or fruit yoghurts.

1.3.2.1. Standardization of milk

The standardization of milk in the production of yoghurt helps to achieve the qualitative requirements of the finished product. It mainly concerns total solids as well as the protein and fat content. Total solids are generally higher for set yoghurts than for stirred yoghurts. Enriching milk with proteins
(to around 5 g per kg$^{-1}$) contributes to the firmness of the gel and prevents the risk of phase separation. This is achieved either by the addition of powder (skimmed milk powder, whey protein concentrate powder), evaporation or membrane technology (ultrafiltration, reverse osmosis).

In addition, carbohydrates such as sucrose or glucose are often added to sweetened or fruit yoghurts. Polysaccharides (pectin, xanthan, etc.) can also be used as stabilizers in fruit yoghurts.

### 1.3.2.2. Homogenization

The homogenization of milk used for fermentation has a number of objectives: it improves the firmness of the gels obtained after fermentation, increases their water retention capacity and reduces syneresis. It also prevents creaming during yoghurt production, in particular during the static incubation period in containers or fermentation tanks. Homogenization is usually carried out in the rising phase of pasteurization at a pressure of around 20 MPa and a temperature between 60 and 90°C. During homogenization, the lipid interface is covered with proteins (casein micelles, whey proteins). The protein coating of homogenized fat globules is involved in the formation of the protein network during acidification [LUC 98].

### 1.3.2.3. Heat treatment

By modifying the physicochemical properties of proteins, the heat treatment of milk (around 90°C/10 minutes) has a significant impact on the rheological properties of lactic gels. Through heat denaturation, whey proteins (more than 90%) form soluble covalent aggregates or aggregates bound to κ-casein on the surface of casein micelles. By changing the micelle surface, heat treatment causes an increase in the pH of acid gelation of milk, an increase in gel firmness and a reduction in its syneresis (Figure 1.11). Furthermore, heat treatment creates a favorable environment for the growth of lactic acid bacteria by destroying undesirable microorganisms and potential competitors to lactic acid bacteria, lowering the redox potential, contributing to the production of formic acid, and so forth.

### 1.3.2.4. Fermentation

After heat treatment, milk is cooled to between 40 and 45°C and inoculated with starter culture, resulting in acidification in either a tank or individual containers. In the case of yoghurt, starter cultures include *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. They grow synergistically
(Figure 1.12) and can be differentiated by their optimum growth temperature, but also by their acidifying capacity and flavor production. Thus, the proportion of strains added during inoculation and the incubation temperature determine the sensory properties of the products. In addition, some strains release exopolysaccharides into the medium, which affect the rheological properties of the gel.

**Figure 1.11. Diagram of the influence of heat treatment on the protein constituents of milk and rheological properties (G') of the gels obtained**

![Diagram showing the influence of heat treatment on milk proteins and gel properties.](image)

**Figure 1.12. Synergistic action of starter cultures in yoghurt**

![Diagram illustrating the synergistic action of Lactobacillus bulgaricus and Streptococcus thermophilus on milk fermentation.](image)
During acidification, casein micelles covered with whey protein aggregates are destabilized and begin to associate when the pH of the medium drops below 5.5. This results in molecular rearrangement, leading to the formation of a gelled protein network that includes homogenized fat globules. The firmness of the network increases with the degree of acidification [TAM 99]. When the pH reaches 4.6, yoghurt is cooled to around 5°C in order to control the metabolic activity of the starter cultures. While set yoghurts (container fermentation) are cooled to 5°C in a single stage, stirred yoghurts (tank fermentation) are cooled in two stages. In the first stage, carried out in a plate heat exchanger, the yoghurt is cooled to 15–20°C. After stirring and smoothing, the yoghurt is then poured into containers and cooled to 5°C.

1.3.3. Milk powder

1.3.3.1. Drying of milk

After bactofugation to eliminate dispersed elements (butyric acid bacteria spores, casein fines), whole, skimmed or standardized milk is heat-treated before drying; it can also undergo different concentration operations (microfiltration, ultrafiltration, nanofiltration) that modify the ratio between milk components. Some components (polysaccharide, minerals, vitamins, etc.) can be dispersed in milk. After standardization, the liquid is homogenized, concentrated by vacuum evaporation and finally spray-dried or drum-dried (see Volume 2).

The concentration of milk and its derivatives by vacuum evaporation (the process of removing water by boiling) is based on lowering the boiling point of the liquid (and therefore the processing temperature) by reducing pressure. Vacuum is used for two main reasons: on the one hand, the temperature difference between the dairy product to be concentrated and the heating surface of the falling film evaporator is greater for a given heating steam pressure, which can reduce steam consumption by increasing the evaporation capacity and/or using more effects; on the other hand, it can evaporate heat-sensitive solutions. The most common apparatus in the dairy industry is the multiple effect evaporator, which incorporates a falling film evaporator equipped with thermal vapor recompression and mechanical vapor recompression systems. The energy cost of removing 1 ton of water is between 360 and 1080 kWh. The maximum boiling at the beginning of the cycle (first effect) is normally less than 70°C, corresponding to an absolute pressure of 30,664 Pa. The evaporation capacity of industrial evaporators
varies from 10 to 30 tons h\(^{-1}\). A concentration cycle lasts between 10 and 20 h. Theoretically, the average residence time of the product in an industrial evaporator is between 10 and 20 minutes: fouling of the evaporation tubes due to the precipitation of calcium phosphate causes a gradual increase in temperature throughout the entire evaporation unit of 10 – 15°C.

**Figure 1.13.** Processing diagram for the production of low-heat skimmed milk powder

The concentrate obtained by vacuum evaporation can be dried using various drying techniques, which are differentiated according to energy cost and powder quality; the most common method is spray drying, which involves spraying the product (liquid or suspension) into a hot gas stream so as to obtain a powder almost instantaneously. It is thus a form of entrainment drying where air acts both as a heater and a carrier for the water removed from the concentrate: the air is dry and hot when entering the drying tower and moist and cooler when it leaves. In Europe, industrial drying systems usually have an evaporation capacity of 0.5 – 4.5 tons h\(^{-1}\), requiring air flows of \(1 \times 10^4\) to \(12 \times 10^4\) m\(^3\) h\(^{-1}\), and production cycles varying from 4 – 24 h on average. Operating a skimmed milk drying facility involves the control of many thermodynamic, physical and technological parameters, such as incoming and outgoing air temperatures (180 – 280°C and 80 – 90°C respectively), velocity and relative humidity of the air (5 – 10% for outgoing air), temperature and viscosity of the concentrate, spray nozzle type (high pressure, two-fluid or turbine), type of tower (1–3 stages) and so on. Under these conditions, it is possible to obtain a skimmed milk powder with \(a_w\) of 0.2 (25°C) and 4% residual moisture. Figures 1.13 and 1.14 are diagrams showing the production
of low-heat skimmed milk powder and the production of reduced whey protein content skimmed milk powder prepared by combining microfiltration and ultrafiltration.

![Processing diagram for the production of skimmed milk powder by microfiltration and ultrafiltration (Primin® powder)](image)

### 1.3.3.2. Physical properties of milk powders

The quality of milk powder depends on many factors, including milk quality before drying and the implementation of the drying process itself. [PIS 81] and [MAS 91] classified the main properties of milk powders into two categories (Figure 1.15):

- properties inherent to the product (biochemical, microbiological, etc.);
- properties inherent to the process (functional properties and any defects).
Particle size

Particle size, determined by particle size analysis, determines several physical and functional properties (flow, density, solubility, wettability, etc.). Dry particle size is mainly influenced by the size of droplets during spraying.

Density

High-density powders can reduce transport costs. The density of milk powders is a complex property that depends on primary factors such as the true or absolute density of the product, the amount of occluded air in each particle and the amount of interstitial air between each particle. Bulk density is mainly influenced by the properties of the concentrate (dry matter, temperature, intensity of the heat treatment, foaming capacity), the drying air (thermodynamic properties at the entry and exit of the facility) and the powder (particle size, residual moisture content).

Hygroscopicity

The hygroscopicity of a powder is characterized by its final moisture content after equilibration with air of controlled relative humidity under
defined temperature conditions. A powder is assumed to be non-hygroscopic if its hygroscopicity percentage is less than 10%. The hygroscopicity of a milk powder is determined by the hydrophilic nature of the components (mainly lactose and amorphous minerals). Reducing particle size, by increasing the powder surface in contact with air of controlled relative humidity, promotes water adsorption and the hygroscopicity of the powder.

**Flowability and floodability**

The ability of a powder to flow has a significant impact on storage, discharge, weighing, mixing, compression, transfer and so on. Carr’s method [CAR 65] is used to determine two types of behavior: flowability and floodability. Flowability involves measuring the angle of repose, the angle of spatula, cohesion and compressibility. Floodability involves measuring the angle of fall, the angle of difference, dispersibility in the air and the value of the flowability index. The two main factors affecting the flowability of powders are particle size distribution and the state of the particle surface. Powders produced using spray nozzles have a higher level of flowability compared to powders produced using heated rollers. Two-stage drying also yields better flow results compared to single-stage drying. Other factors that improve flowability are powder agglomeration, a low level of fines, the addition of a flow agent (silica), the addition of hygroscopic compounds (carbohydrates, whey) and low levels of free fats.

1.3.3.3. **Technological properties of milk powders**

**Rehydration properties**

The ability of a milk powder to rehydrate in water is an essential property for industrial users of dried ingredients and can be characterized by three properties: wettability, dispersibility and solubility. They depend on powder composition and the affinity between these components and water, and the accessibility of water in terms of structure (porosity and capillarity) to the powder components.

Wettability, the ability of a powder to immerse itself once placed on the surface of water, reflects the capacity of powder to absorb the water on its surface. The swelling ability (swellability) of a powder is also linked to wettability. The structure of a powder disappears when the various
components (in particular proteins) are dissolved or dispersed. Factors influencing wettability include:

- the presence of large primary particles, such as agglomerated particles: this is a desired effect with the granulation (with or without recycling fines) of milk powders;
- powder density;
- the presence of fat on the surface of powder particles (free fats);
- porosity and capillarity of powder particles as well as the presence of interstitial air.

Dispersibility is probably the best individual criterion for assessing the rehydration ability of a milk powder, since to a certain extent, it is influenced by wettability and solubility. Dispersibility is improved by:

- a decrease in protein content;
- an optimal particle size of 200 µm;
- drying at low temperatures (low heat powder).

The insoluble materials formed during the production of milk powder are usually due to the denaturation of soluble proteins and the precipitation of calcium phosphate. Thus, solubility is particularly influenced by heat treatment before drying, the viscosity and biochemical composition of the concentrate, the drying air temperature and the particle size of the powder.

**Use of recombined milk in cheese processing**

The use of recombined milk from powder is justified for several reasons: for economic, nutritional, dietary and geographical purposes, and also for sensory and technical purposes. It allows the transfer of cheese production to countries where milk production is insufficient, and where milk production has a high seasonality (in the case of goat’s or sheep’s milk).

Milk powders used in cheese production must have a level of microbiological quality that is in compliance with regulations and acceptable for cheese making. These factors depend on the initial quality of the milk used and the intensity of the heat treatments during processing into powder, which are the source of physicochemical changes resulting in reduced coagulation properties when milk powder is reconstituted with water. In order to meet
these microbiological and technological requirements, HTST (pasteurization at 75°C for 20 s) is recommended prior to drying in order to ensure hygienic quality while maintaining a high level of coagulation. These recommendations only apply if the milk is of good microbiological quality. Otherwise, the intensity of the heat treatment must be higher, therefore compromising coagulation properties: in this case, the milk powders obtained cannot be used in the production of cheese.

Cross-flow microfiltration (1.4 µm) followed by vacuum evaporation at low temperature and spray drying is well suited for the production of an “ultra-low-heat” powder (low level of denaturation of soluble proteins); milk reconstituted from this powder, according to regulatory microbiological requirements, has the same level of rennet coagulation as the original raw milk ([SCH 94], Figure 1.16).

The technological quality of a powder intended for cheese production can be improved by reducing the soluble protein content of the milk. Native phosphocaseinate (NPC) powder can be produced using 0.1 µm cross-flow
microfiltration (see section 9.1). The rennet clotting time of NPC reconstituted to 3% from powder is reduced by 53% and the firmness of the rennet gel after 30 min is improved by 50% compared to raw milk at the identical casein concentration. Enriching milk with milk microfiltrate (0.1 µm) can significantly improve cheese yield, especially in the case of hard cheeses. In addition, the partial removal of soluble proteins that otherwise aggregate on the surface of casein micelles on heating, limits the negative effects of heat treatments on rennet coagulation. These factors have led to the development of technology for producing medium- or high-heat powder (e.g. Primin®), which has similar or even greater suitability for cheese production compared to raw milk (Figure 1.17).

![Figure 1.17. Processing diagram for the production of Primin milk powder (VRF = volume reduction factor)](image)

1.3.4. Cheese

Cheese making is an ancient way of preserving milk (protein, fat and some calcium and phosphorus). Its nutritional and sensory qualities are valued in almost every part of the world.
The name “cheese” is reserved for a fermented or non-fermented, ripened or non-ripened product of exclusively dairy origin (milk, partially or fully skimmed milk, buttermilk) used alone or as a mixture. It is totally or partially coagulated before draining or after partial removal of water. Cheese may be considered a concentration of the major components of milk (protein, fat), produced by draining curd obtained by acidification and/or enzymatic action (usually rennet extracted from the stomach of a calf before weaning). Cheese production involves four phases: milk standardization, coagulation, draining and ripening (Figure 1.18).

![Figure 1.18. Steps of cheese production](image)

The preparation of milk (standardization) for a given cheese relies on physicochemical and microbiological “standards”. Transformation from the liquid state to the gel state (coagulation) differs depending on whether coagulation is induced by acidification and/or enzymatic (rennet) action. After phase separation (draining), the curd may undergo a ripening process specific to each type of cheese.

A wide variety of cheeses can be produced using traditional technologies depending on the type of milk used (cow’s, goat’s or sheep’s milk – alone or mixed), the pH of coagulation and the relative kinetics of acidification and whey removal (draining) from the curd.
1.3.4.1. Physicochemical and biological standardization of milk

The quality of milk for cheese production can be defined by its suitability to form a coagulum resulting, after draining and eventually ripening, in a cheese with defined physicochemical properties and a satisfactory yield. Milk has a varied composition depending on the animal species, breed, individual, lactation stage and number, method and time of milking, season, climate, diet and so forth. Not all milk has the same suitability for cheese production since it differs in some characteristics such as casein content and composition, salt balance, lactose content, hygienic quality, pH and so on. These characteristics affect their ability to coagulate, which is necessary to pass from the liquid to the solid state, as well as the properties of the coagulum.

In order to avoid variations in the protein content of milk and improve coagulation properties, which affect cheese yield and quality, manufacturers are able to adjust the milk protein level to between about 30 and 42 g L\(^{-1}\) using various techniques: removal of water by evaporation or reverse osmosis, concentration by nanofiltration, ultrafiltration (most common), microfiltration or the addition of caseinates.

In order to adjust the “fat/dry matter” ratio that is specific to each type of cheese, manufacturers standardize the milk fat while taking into account the milk protein composition. Using a weight of standardized milk \((w_{SM})\) in terms of fat \((F_{SM})\) and protein \((P_{SM})\), and knowing the cheese yield and recovery coefficients of these constituents in the cheese (see section 1.3.5.3), it is possible to obtain a weight of cheese \(w_C\) with the desired characteristics (fat content \(F_C\), protein content \(P_C\); Figure 1.19).

![Figure 1.19. Standardization of the fat and protein content of milk intended for cheese production](image-url)
To correct for variations in the calcium content of milk during the lactation stage or changes in the calcium balance between the soluble and colloidal phase due to the effects of refrigeration or heat treatment, manufacturers add CaCl₂ at a dose ranging usually from 80 to 200 mg L⁻¹ of milk, which improves the coagulation properties of the milk.

To meet the processing time requirements (rennet clotting time, rate of curd formation, hardening time) and the desired mineral content in the curd, which depend on the type of cheese desired, manufacturers adjust the pH rennet added into milk by fermentation (lactic starters), the addition of glucono-δ-lactone, the injection of CO₂, or the addition of acid whey proteins.

For certain types of cheeses, the lactose content of milk is lowered by washing the curd (in medium-hard cheeses) or by ultrafiltration of the milk followed by diafiltration before coagulation (in “stabilized” soft cheeses). The partial removal of lactose slows down the activity of lactic bacteria and is a mean of controlling the pH of the curd at the end of the acidification.

Biological standardization of cheese milk consists of the elimination of the endogenous microorganisms in the milk that may be undesirable (psychrotrophs, pathogens) by heat treatment, bactofugation or microfiltration, followed by the addition of a controlled starter culture; prematuration at a low temperature (10–12°C, 12–18 h) by promoting the production of growth factors, improves the lactic fermentation process.

1.3.4.2. Coagulation

There are three types of coagulation (Figure 1.20).

**Acid coagulation**

Acid coagulation involves the precipitation of casein at its isoelectric point (pI = 4.6) by biological acidification using lactic acid bacteria to transform lactose to lactic acid, or by chemical acidification (injection of CO₂, addition of glucono-δ-lactone or addition of acid whey proteins). The chemical method (organic acid) is mainly used to standardize the pH of milk before renneting, while the addition of mineral acid is usually not permitted.

Gel formed by the solubilization of colloidal calcium phosphate during acidification has good permeability but high friability; the lack of structure in
the network (low energy hydrophobic interactions) results in almost zero elasticity and plasticity as well as low resistance to mechanical treatment.

Enzymatic coagulation

Enzymatic coagulation is used to transform milk from a liquid to a gel state through the action of proteolytic enzymes, mostly of animal origin.

There are three phases:

– primary or enzymatic phase, corresponding to the hydrolysis of $\kappa$-casein at the bond between phenylalanine (105) and methionine (106);

– secondary phase or aggregation of hydrolyzed micelles, which, at pH 6.6, begins when 80 – 90% of the $\kappa$-casein is hydrolyzed;

– tertiary or cross-linking phase leading to gel formation.
Several factors influence coagulation, such as enzyme concentration, temperature, pH, calcium content, casein composition, micelle size and pre-treatment of milk such as cooling, heat treatment and homogenization.

The network formed at pH 6.6 is rich in minerals, given the numerous interactions between calcium and casein at this pH; this type of gel tends to contract, which leads to an expulsion of whey.

**Mixed coagulation**

Mixed coagulation results from the combined action of rennet and acidification. The variety of combinations, resulting in casein micelles with different mineral content when gelation occurs and whey is drawn off the curd, is the source of a wide range of soft cheeses, semi-soft cheeses and medium-hard cheeses.

### 1.3.4.3. Draining

**Lactic acid and rennet gels**

The draining stage involves removing some of the whey trapped in the gel network formed by acidification and/or enzymatic action. It begins in the coagulation tanks and continues in the moulds and finally in the cheese-ripening rooms. It is possible to express the whey flow rate based on Darcy’s law (see Chapter 3, Volume 2):

\[
\dot{V} = \frac{A \Delta P}{R \eta}
\]

where \( \Delta P \) is the differential pressure exerted on the gel (Pa), \( \eta \) is the viscosity of the whey (Pa s), \( R \) is the hydrodynamic resistance of the gel (m\(^{-1}\)) and \( A \) is the surface area of the gel (m\(^2\)).

As a result, whey removal depends on:

- the type of curd, which affects permeability in particular (\( \frac{1}{R} \) term of [1.4]); gel porosity decreases during acidification (increase in \( R \)), but this is offset by the reduced water retention capacity of protein close to its pI;

- extent of the mechanical and thermal treatments applied on the curd in the tank, which involves slicing the curd (increase in \( A \)), subsequent stirring to
avoid sticking (maintaining $A$) and heating (decrease in $\eta$; energy supply strengthens the protein network and contractability of the gel resulting in an increase in $\Delta P$);

– the pressing stage after molding (increase in $\Delta P$), which removes the remaining whey and strengthens the cohesion of the curd in the case of hard cheeses.

The kinetics of the removal of whey from the mould can be described by equation [6.9] (see Chapter 6, Volume 2). In this case, flow resistance increases due to the obstruction of the mould perforations by the curd grains, making it necessary to turn the cheese regularly so as to promote draining.

The natural drainage of a lactic gel is slow and limited. It results in a heterogeneous curd with a low dry matter and mineral content: the weakly cross-linked network contracts only slightly. Processes such as centrifugation or ultrafiltration of the curd can significantly increase drainage compared with traditional methods (strainer, bag or filter drainage). Subjecting milk and/or acid gel to intense heat treatment (80–95°C for several minutes) can increase cheese yield by denaturation and retention of whey proteins. However, heat treatment, as well as homogenization, limits the rate and intensity of drainage.

Rennet gel has strong cohesion, elasticity and porosity, but low permeability leading to limited natural drainage. As a result, it is necessary to carry out different operations in the tank (slicing, mixing, slow and steady heating up to 56°C for hard cheeses) to allow drainage of the gel. The higher the dry matter content required, the more intense these processing steps become; however, they also reduce cheese yield and the recovery coefficients of the cheese components.

**Mass balance, cheese yield and recovery rate**

When processing milk into cheese, it is possible to calculate the mass balance for a constituent $X$ (protein, fat, etc.) using the following equation:

$$\begin{cases} w_M X_M = w_C X_C + w_W X_W \\ w_M = w_C + w_W \end{cases}$$

[1.5]
where \( w_M, w_C \) and \( w_W \), and \( X_M, X_C \) and \( X_W \), respectively, are weight (kg) of milk, cheese, whey, and concentrations of the constituent \( X \) in milk, cheese and whey (g kg\(^{-1}\)). Cheese yield \( Y_C \) (dimensionless) is expressed in kg of cheese per 100 kg of milk used.

\[
Y_C = \frac{w_C}{w_m} \times 100 \quad [1.6]
\]

By combining [1.5] and [1.6], we get

\[
Y_C = \frac{X_M - X_W}{X_C - X_W} \times \frac{100}{100} \quad [1.7]
\]

For example, if 50 kg of milk with 32 g kg\(^{-1}\) of protein give 6.7 kg of cheese and 43 kg of whey with 185 g kg\(^{-1}\) and 8.5 g kg\(^{-1}\) of protein respectively, the cheese yield \( R_F \) is equal to 13.3%. To standardize the cheese yield calculation, technologists often calculate the yield of a reference cheese; this corrected yield (\( Y_{CC} \)) is:

\[
Y_{CC} = \frac{X_M - X_W}{X_{\text{ref}} - X_W} \times \frac{100}{100} = Y_C \frac{X_C - X_W}{X_{\text{ref}} - X_W} \times 100 \quad [1.8]
\]

where \( X_{\text{ref}} \) is the concentration of constituent \( X \) in a reference cheese, representative of a given technology.

For a given manufacturing process, it is also possible to calculate the recovery rate \( R_X \) of a constituent \( X \) in cheese based on the following equation:

\[
R_X = \frac{w_C X_C}{w_M X_M} \times 100 \quad [1.9]
\]

In the previous example (50 kg of milk with 32 g kg\(^{-1}\) of protein gives 6.7 kg of cheese with 185 g kg\(^{-1}\) of protein), the recovery rate of protein \( R_{\text{prot}} \) is therefore 77.5%.

**Drainage and acidification kinetics: categories of cheese**

The physicochemical properties of cheese during demolding (fat-free dry matter – FFDM, fat content, \( \text{pH} \), moisture in non-fat substance – MNFS,
calcium on a from basis – Ca/FFDM), which determine the ripening process by influencing microbial growth and biochemical and enzymatic reaction kinetics, depend on the intensity and relative position of the drainage and acidification stages (Figure 1.21).

MNFS (dimensionless), which expresses the availability of water in the curd, is calculated as follows:

\[
\text{MNFS} = \frac{100 - \text{DM}_C}{100 - \text{F}_C} \times 100
\]

[1.10]

where \( \text{DM}_C \) and \( \text{F}_C \) are the dry matter and fat content of cheese, respectively.

There are four main categories of cheese:

– *Acid curd*, such as fresh cheeses: This is high-moisture cheese; in this case acidification of the milk substrate precedes drainage. Whey is drawn off at acid pH (4.5–5), under conditions where more than 80% of calcium and phosphates are solubilized in the whey. This leads to a significant demineralization of the cheese, which accentuates its friability and crumbliness.

– *Rennet curd*, such as semi-hard and hard cheeses (cooked pressed cheese): Processing involves intense drainage after rennet coagulation. Drainage therefore precedes acidification that occurs in a lactose-depleted medium; the buffering capacity is largely due to the concentration of proteins and minerals (FFDM up to 30–35%). As a result, the pH of the cheese at the end of the acidification stage is generally between 5.2 and 5.4, and its calcium content is higher than other types of cheeses (2.9 < Ca/FFDM < 3.1%). These characteristics give an elastic and cohesive texture; the low level of MNFS results in a shelf life of several months.

– *Mixed curd with a predominantly acid nature*, such as traditional and industrial soft cheeses: These are high-moisture cheeses (MNFS around 75%), relatively acidic before ripening (pH 4.6–4.8) and depleted of minerals. The shelf life of these products is no more than a few week.

– *Mixed curd with a predominantly rennet nature*, such as semi-soft cheeses and medium-hard cheeses (stabilized soft cheese, uncooked or semi-cooked pressed cheese): There is a greater level of drainage compared with
the previous category, which may involve a lactose-removal stage. The pH at the end of the acidification stage ranges from 4.8 to 5.2 and the cheese has a moderate mineral content. The shelf life is a number of weeks depending on the MNFS (60 – 72%).

![Drainage and acidification kinetics](image)

**Figure 1.21. Drainage and acidification kinetics and processing types (according to [MIE 91])**

The following Figures (1.22, 1.23, 1.24 and 1.25) give examples of processes associated with the production of acid curd (fresh cheese; Figure 1.22), mixed curd with a predominantly acid nature (industrial Camembert; Figure 1.23), mixed curd with a predominantly rennet nature (Saint-Paulin; Figure 1.24) and rennet curd (Beaufort; Figure 1.25).

**Decoupling of drainage and acidification kinetics**

It is possible to completely decouple drainage and acidification kinetics by concentrating milk proteins and fat by means of cross-flow filtration. Ultrafiltration (see Chapter 6, Volume 2) can concentrate all the milk proteins,
whereas 0.1 µm microfiltration results in the total retention of caseins and the transmission of whey proteins to the permeate (transmission rate between 60 and 80%); as a result, 0.1 µm microfiltration is mainly carried out when the cheese manufacturer also looks for a high-quality permeate, which in this case is a “true whey” (absence of phospholipids, casein fines and any particles).

**Figure 1.22. Manufacturing process of fresh cheese**
Figure 1.23. Manufacturing process of industrial Camembert
Figure 1.24. Manufacturing process of Saint Paulin
Figure 1.25. Manufacturing process of Beaufort

The ultrafiltration of milk results in a liquid pre-cheese, which has the composition of drained cheese [MAU 69]. This process eliminates drainage after coagulation and therefore allows, in some cases, molding directly into the containers for retail; it reduces the weight difference due to molding of the curd, increases yield (by 10 – 20% through whey protein retention in the cheese) and decreases rennet consumption (added after filtration). It has, however, the disadvantage of yielding cheeses with high lactate and lactic acid.
contents, due to the high buffering capacity of pre-cheeses resulting from the concentration of calcium phosphate associated with casein. To overcome these problems, it is possible to pre-acidify and salt the milk to solubilize some of the colloidal calcium phosphates, which are then removed during ultrafiltration. This method is limited, however, since pre-acidified milk is thermally less stable and it is more difficult to add value to the permeate.

1.3.4.4. Ripening

Ripening involves the enzymatic digestion of the protein and lipid components in the curd. It is a complex biochemical process for several reasons:

– the cheese matrix resulting from the coagulation and drainage of milk has a very high level of physicochemical heterogeneity;

– enzymes involved in ripening have several origins: they could be endogenous milk enzymes (plasmin, lipase, etc.), added to milk during manufacturing (coagulating enzymes, microorganisms) or produced during ripening by microbial synthesis (bacteria, yeasts, moulds).

The curd and biological agents constitute a complex ecosystem and a heterogeneous bioreactor, the parameters of which are not always well defined. Ripening is dominated by three major biochemical phenomena:

– the fermentation of residual lactose and the degradation of lactate;

– the hydrolysis of fat and proteins;

– the production of aroma from fatty acids and amino acids.

These transformations give the cheese its characteristics; they modify its appearance, composition and consistency, while at the same time flavor, aroma and texture develop.

Substrates

The physicochemical properties of the curd vary with the manufacturing process. While the composition of the curd is well defined, its structure is more complex. It is difficult to control microbial growth and enzymatic action in such a complex and heterogeneous medium.

The kinetics of ripening depends on the mobility of carbohydrates, proteins and lipid substrates, reaction products (lactate, amino acids, fatty acids) in the
cheese matrix, and the rate of biological reactions, which depends on the pH of
the matrix and its water availability. Reactions are generally faster when the
pH of the curd is close to neutral (optimal activity pH of flora and enzymes)
and the MNFS is high. The shelf life of the product depends on the buffering
capacity of the cheese, which limits and regulates the increase in pH
(alkalinization) of the cheese during ripening (rennet curd and mixed curd with
a predominantly rennet nature).

**Ripening agents**

The enzymes involved in ripening have several origins: milk, coagulating agent and microorganisms in the cheese.

**Milk enzymes**

− *Plasmin*: heat resistant protease active in slow-ripened cooked and
uncooked pressed cheeses.

− *Alkaline phosphatase*: denatured by pasteurization, it is active in raw
milk cheeses only.

− *Lipase*: thermolabile enzyme active in raw milk cheeses only. It
hydrolyzes short chain fatty acids in particular. Its action is more pronounced
in goat’s and sheep’s milk because the proportion of short-chain fatty acids is
higher and the fat globules are smaller than those of cow’s milk.

**Coagulating enzymes**

Rennet (mixture of chymosin and pepsin), a coagulating agent added to
milk, has a wide spectrum of proteolytic activities. Its action is dominant in
uncooked pressed cheeses. The reaction products formed are mainly high-
molecular-weight peptides.

**Enzymes of microbial origin**

These enzymes come from five main microbial groups:

− *Lactic acid bacteria*: present in the starter culture, convert lactose into
lactic acid. They include:

  - *lactococci*: dominant flora in uncooked soft and pressed cheeses; they
produce lactic acid and exhibit proteolytic activity,

  - *thermophilic streptococci and lactobacilli*: flora in cooked pressed
cheeses; they exert acidifying and proteolytic activity,
- *Leuconostoc*: they produce aromatic components in addition to lactic acid and contribute to the open texture of blue cheese.

- *Propionic bacteria*: produce propionic acid from lactate, are responsible for the open texture of cooked pressed cheeses and contribute to the formation of flavor and aroma.

- *Surface bacteria*: the most common are micrococci and coryneform bacteria (*Bacterium linens*); they are present in washed-rind and smear-ripened soft cheeses. They exhibit proteolytic and lipolytic activity.

- *Yeasts*: the most common is *Geotrichum candidum*; it grows on the cheese surface by consuming lactic acid, producing ethanol and exhibiting lipolytic and proteolytic activity.

- *Molds*: the two most common are *Penicillium camemberti*, which is a surface mold on bloomy rind cheese, and *Penicillium roqueforti*, an internal mold in blue cheeses. They have the most lipolytic enzymes, are responsible for the formation of methyl ketones and secondary alcohols, and also exhibit proteolytic activity.

**Influence of ripening on the flavor of cheese**

The development of flavor and aroma in cheese is based on a number of changes that occur during ripening (Figure 1.26). Several components, from various classes, are involved in this process (acids, alcohols, esters, sulfur products, etc.). Most of these compounds are found in all cheeses but in varying quantities and proportions:

- in fresh cheeses, the flavor is based on acidity and acetaldehyde, which contributes to the fresh character of the cheese;

- in bloomy rind soft cheeses (Camembert), the main compounds are 1-Octen-3-ol, methyl ketones, secondary alcohols, phenolic compounds (phenylethanol and its esters), and various garlic-smelling volatile sulfur compounds;

- in washed-rind soft cheeses, (Limburger, Munster), surface bacteria (corynebacteria and micrococci) degrade amino acids and volatile fatty acids into sulphur compounds (methanethiol and thioesters);

- in blue cheeses, there is a high proportion of free fatty acids, methyl ketones, secondary alcohols and lactones;
– in semi-hard cheeses (cheddar), some authors attribute the aromatic base note to short-chain fatty acids (C₂ to C₆), methyl ketones and corresponding alcohols;

– in hard cheeses, where the level of proteolysis is high, the flavor is created by amino acids, acetic acid, propionic acid, alcohols, esters and sulfur products.

Figure 1.26. Change in constituents during ripening

1.3.4.5. Errors and defects

Given the diversity and complexity of cheese processing, manufacturers must face the risk of errors resulting in defects in the final product. These defects can be classified into two categories: coagulation and draining defects and ripening defects.

Coagulation and drainage defects

The growth of lactic acid bacteria plays a key role in cheese technology. The bacteria act as an acidifying agent, and therefore contribute to coagulation, drainage and the adjustment of the degree of mineralization in the cheese.

The ability of milk to allow the growth of lactic acid bacteria varies with the origin of the milk and the bacterial species. Milk contains a number of
natural inhibitors (immunoglobulins, lactoperoxidase, lysozyme, lactoferrin, nisin, free fatty acids, leukocytes, etc.) and stimulants such as growth factors (group B vitamins, amino acids, nitrogenous bases, small peptides, proteose peptones). Subjecting milk to heat treatment may destroy natural inhibitors and growth factors, but it can also generate growth factors such as peptides, amino acids, formic acid and so on. Other exogenous factors such as bacteriophages, antibiotics or chemical residues can inhibit the growth of lactic acid bacteria.

Finally, coagulation defects (longer rennet clotting time, slower firming rate, formation of a soft gel with reduced cheese yield) may occur in milk due to its physicochemical and bacteriological composition (mastitis milk, milk from the beginning or end of lactation) or the type of treatment it has undergone (refrigeration, heat treatment, etc.).

Ripening defects

Ripening defects can be classified into three categories:

– *texture and swelling defects*: these defaults can be caused by processing (dry, oily or runny rind, split in the body of the cheese, untypical number, size and uniformity distribution of eye in certain semi-hard cheese, etc.) or microbiological reasons (early or late blowing, off-odor);

– *appearance defects (crust texture, undesirable mould growth)*: these can be caused by fungus on the cheese surface (“blue”, “cat hair” or “toad skin” defects), or fungus and bacteria on the surface and inside the cheese (cheese rind rot, mottled appearance with flecks of orange, cream, pink, brown, white, red, etc.);

– *flavor and aroma defects*. These include:

  - *bitter flavor*, frequently encountered in pressed, blue and soft cheeses. Caseins (mainly hydrophobic β-casein) are responsible for the formation of bitter peptides by the action of residual rennet, plasmin, penicillii, psychrotrophic bacteria and some starter cultures that acidify the curd rapidly,

  - *rancid flavor*, which occurs with excessive lipolysis during ripening causing a large amount of short and medium chain-free fatty acids to form. The agents responsible are certain penicillii, psychrotrophic bacteria, natural or microbial lipases (contamination, heat-resistant enzymes, starter cultures, etc.),
- other flavor defects including cruciferous vegetable, mushroom, potato, or malt odors among others. The origins and mechanisms of their formation are diverse and difficult to establish.

In conclusion, the preparation of milk is an important step because it plays a key role in the production of cheese. With the increase in scientific knowledge over the past 30 years, the various stages of processing milk into cheese have become better controlled: the biological, biochemical, chemical, and physical properties of products are constantly changing, demonstrating a vast variety and complexity of reactions, in particular during ripening. However, greater understanding of the physicochemical and microbiological mechanisms involved in the various stages of cheese production is needed.

### 1.3.5. Cream and butter

Cream and butter are dairy products with a higher fat content than milk; except for low-fat products, the fat content of cream and butter, respectively, is greater than 30% and at least 80%. Thus, the production of cream and butter begins with the separation of the fat globules from the skimmed milk. This is achieved by centrifugal separation in hermetic separators with conical plates (see Chapter 3, Volume 2) at a temperature usually ranging between 45 and 55°C.

#### 1.3.5.1. Cream

Cream can be liquid (whipping cream), thick (sour cream) or aerated (whipped cream). Liquid cream has either undergone pasteurization (“crème fraîche”) or UHT sterilization. Thick cream is obtained after inoculating a pasteurized cream with certain starters. Whipped cream is obtained by introducing air into pasteurized or sterilized cream at low temperatures (usually between 4 and 10°C).

**Homogenization and heat treatment**

After separation, cream is homogenized to improve storage stability (creaming in liquid creams, release of whey in thick cream, etc.) or functionality (viscosity of cream, stability for cooking, foaming capacity, etc.). The concentration of available proteins in cream (casein, whey protein) for interface creation during homogenization is often limited. As a result, they are located at the interface of adjacent fat globules, leading to aggregate formation conducive to creaming. To limit this phenomenon, cream is homogenized
using a two-stage homogenizer. The homogenization pressure of the first stage, between 13.5 and 20 MPa, is used to create the interface and decrease fat globule size; the second stage, set at a homogenization pressure of 10 – 20% of that of the first stage, separates the aggregates of fat globules formed during the first stage.

Cream is heat-treated in plate heat exchangers (before or after the homogenization); the exchange area is about three times larger than that used in the treatment of milk due to the lower heat transfer coefficient in cream. In addition, temperatures are higher generally due to higher microbial load in cream and to the high thermal resistance of microorganisms in the presence of fat: the intensity of the heat treatment increases with increased fat content. In the case of pasteurization, the time/temperature combination is approximately 50–10 s/80–100°C. Heat treatment is particularly challenging, since cream is a fragile emulsion and rapid temperature variations can significantly alter the properties of the emulsion.

**Ripening**

Ripened or sour cream is a thick cream. Pasteurized cream is inoculated with up to 0.5% starter culture consisting of a combination of acidifying, aromatic (*Lactococcus lactis* subsp. *Lactis cremoris*, *Lactococcus lactis* subsp *Lactis diacetylactis*, *Streptococcus thermophilus*) and sometimes thickening strains (*Leuconostoc*), which produce exopolysaccharides generating thick creams at less acidic pH. The ripening phase takes 12 – 18 h at temperatures between 12 and 22°C. Acidification causes the gradual destabilization of casein micelles, some of which are adsorbed on the surface of the homogenized fat globules, resulting in the formation of a network of proteins and fat globules, and a thickening of the cream. The most significant changes of texture occur at a pH below 5.0–5.2.

**Whipping**

Whipped cream is a foamed emulsion in which air bubbles are incorporated into a network of partially coalesced fat globules; in the presence of emulsifiers (mono- and diglycerides) and stabilizers (gelatine, carrageenan, etc.), this network ensures the rigidity and stability of the foam.

Cream intended for whipping is first homogenized, which increases the number of fat globules, and heat-treated before being refrigerated (4 – 10°C) for several hours (approximately 20 h) to promote fat globule crystallization.
During the aging of cream at low temperature, emulsifiers gradually displace adsorbed proteins from the homogenized fat globule surface, thereby reducing fat globule stability [GOF 17]. During whipping, the collision of destabilized fat globules promotes partial coalescence. Partially coalesced homogenized fat globules move to the air interface and form a network that stabilizes air bubbles. In addition, stabilizers increase the viscosity of the non-fat phase and limit drainage by interaction with proteins of the non-fat phase and adsorbed proteins on the fat globules.

1.3.5.2. Butter

Butter consists of a continuous liquid fat phase in which triglyceride crystals, small fat globules, aqueous phase droplets and air bubbles are dispersed (Figure 1.27). It is made from cream, typically pasteurized, containing 40 – 50% fat, which is traditionally ripened (cultured butter) and then churned to induce phase inversion. Ripening includes two combined operations:

– fat globule crystallization to develop the rheological properties of butter;
– cream fermentation to develop aroma and decrease the pH of cream.

These combined operations occur in cultured butters obtained by traditional batch churning or a continuous manufacturing process (Fritz process). The manufacture of cultured butters has gradually been replaced by the NIZO method, which is more flexible and economical, whereby fat globule crystallization and the production of flavor and acid are separate.

![Figure 1.27. Structure of butter](image)
**Fat globule crystallization**

Cream storage/aging at low temperature aims to induce the partial crystallization of fat, thereby promoting phase inversion. By strict control of the thermal cycle, it also adapts the consistency of butter to the seasonal and geographical variability in milk fat composition. In practice, there are two types of aging:

- low-temperature aging for winter cream, whereby the cream is immediately cooled to 6–7°C allowing the formation of many small fat crystals;
- high-temperature aging for summer cream whereby temperatures are adapted to obtain large fat crystals.

After aging, the solid and liquid fat ratio in cream is relatively stable.

**Cream fermentation**

Cream fermentation aims to acidify cream, allowing the development of a marked and typical aroma, promote phase inversion by decreasing the surface potential of fat globules at low pH, and ensure biological protection against microorganisms that can degrade butter. The major disadvantage of cream fermentation is that it generates an acidic and aromatic by-product after churning (buttermilk), which is difficult to stabilize and process further. This was the driving force behind the developments in butter technology and the introduction of the NIZO process.

Inoculation of cream with 3–5% lactic acid bacteria is achieved using a dosing pump. It can be performed either at the beginning of cream aging (before fat globule crystallization), resulting in pH values below 5.0, or after fat globule crystallization. Currently, the desired final acidity of butter is significantly less than it was in the past. Fermentation is usually carried out below 15°C for 10 – 12 hours. When the pH reaches a value close to 5.5–5.8, ripening is slowed by cooling the cream to 8°C. Butter produced in such a way has a storage pH ranging from 5.2 to 5.6.

**Phase inversion**

Phase inversion involves transforming ripened cream, an oil-in-water emulsion, into butter, a water-in-oil emulsion. Phase inversion is performed by churning, or vigorous agitation, at a temperature corresponding to the optimum ratio of crystallized and liquid fat (normally 10 – 13°C for churning...
winter cream and 7 – 10°C for summer cream). During churning, air bubbles are incorporated into the cream. The air bubble interface is first stabilized by (non-homogenized) fat globules. When they become insufficient in number to cover the newly-created interface, the foam collapses causing a rapid convergence of fat globules [VAN 01]. Coalescence of fat globules is promoted by the reduction of its surface charge depending on cream acidity and the presence of fat crystals deforming the fat globule surface. The release of liquid fat contained in the fat globules causes an agglomeration of fat crystals, intact fat globules and fat globule fragments in the form of granules. When a sufficient amount of liquid fat has been released, the granules are converted into butter grains in which droplets of buttermilk and small fat globules are dispersed. The emulsion is then rapidly reversed and the buttermilk is expelled. After washing and optional salting, the butter is kneaded to compact the butter grains and ensure homogenization by evenly distributing the aqueous phase and salt.

Conventional churning is carried out batch-wise in a barrel churn rotating about a horizontal axis. It is generally filled to 40–50% of its volume with ripened cream. Rotation ensures the incorporation of air into the cream and phase inversion. The churn has an outlet for releasing butter-cream and wash water. Continuous churns, or butter-making machines, operate according to the same principle as conventional churns, but without interruption. A butter-making machine consists of a cooled cylinder containing a rotating beater that incorporates air resulting in phase inversion and a tilted kneading cylinder containing two counter-rotating augers that compress and release the butter. The butter is generally washed and kneaded under vacuum to limit the risk of oxidation.

1.3.5.3. NIZO butter

The NIZO (Netherlands Institute for Dairy Research) method is used to prepare butter from cream that has not been fermented. Apart from this exception, all other processing stages remain the same as for the continuous manufacture of butter. Acidifying and flavoring agents (NIZO mixture) are added at a rate of approximately 0.8 – 1.25% to sweet butter after kneading. The NIZO mixture is prepared under aerobic conditions by vigorously mixing around 40% of a lactic acid concentrate (lactic acid content of the concentrate close to 18%) obtained by culturing Lactobacillus helveticus in whey, and around 60% of a mesophilic lactic starter (Lactococcus lactis, Lactococcus cremoris and Lactococcus diacetylactis). Intense oxygenation of the mixture is
favorable for diacetyl production, characteristic of the butter aroma. The final mixture is very acidic and no longer contains live bacteria. This first injection is followed by a second injection of live bacterial culture consisting of acidifying strains of *Lactococcus lactis* and *Lactococcus cremoris* as well as a different aromatic strain of the previous culture, *Leuconostoc cremoris*. *Leuconostoc cremoris* is able to consume excess acetaldehyde, responsible for the “yoghurt” taste in the NIZO mixture (Figure 1.28).

There are several advantages to the NIZO method: the by-product generated (sweet buttermilk) is easier to use in further processing; the pH of butter can be adjusted upon injection of the lactic acid concentrate; and the control of starter culture conditions (controlled temperature and culture medium) ensures a high level of regularity in the production of aromas. Finally, the crystallization temperature of fat in cream is determined solely for the purpose of controlling the rheological properties of butter [MIL 98].

**Figure 1.28. Manufacture of butter based on the NIZO method**