FOOD SCIENCE AND TECHNOLOGY SERIES

Handbook of Food Science and Technology 3

Food Biochemistry and Technology

Edited by

Romain Jeantet, Thomas Croguennec
Pierre Schuck and Gérard Brulé

ISTE WILEY

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First published 2016 in Great Britain and the United States by ISTE Ltd and John Wiley & Sons, Inc.
Translated by Geraldine Brodkorb from “Science des aliments” © Tec & Doc Lavoisier 2006.

ISTE Ltd
27-37 St George’s Road
London SW19 4EU
UK

www.iste.co.uk

John Wiley & Sons, Inc.
111 River Street
Hoboken, NJ 07030
USA

www.wiley.com

Library of Congress Control Number: 2016936913

British Library Cataloguing-in-Publication Data
A CIP record for this book is available from the British Library
ISBN 978-1-84821-934-2

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Introduction

The processing into food of raw materials from hunting, gathering, fishing and subsequently arable and livestock farming has always had two objectives: to preserve nutrients in order to defer the time and place of consumption, and develop products with a wide variety of textures and flavors to satisfy the sensory needs of consumers. The development of arable and livestock farming has facilitated an improved control of supply, even though the provision of agricultural products has long remained very irregular due to climatic or health risks and the seasonality of certain products. Furthermore, the importance of stabilization and/or processing has significantly increased with the rural exodus, which has led to a distancing of production from consumption areas.

The production of certain foods that still form the basis of our diet today dates back several centuries or even millennia, as in the case of bread, cheese and wine for example. These products, particularly those derived from fermentation, were developed based on empirical observations, with no knowledge of the raw materials or phenomena involved in their processing. It was not until the work of Pasteur in the 19th Century that microorganisms gained a key role in the development and processing of agricultural products.

The agri-food industry has undergone a major change over the past few decades in order to better meet the quality requirements of consumers; while traditional food is the result of a series of increasingly understood and controlled biological and physicochemical phenomena, this is not the case for a number of new products designed to meet market expectations. These

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Introduction written by Gérard BRULÉ.

products are the result of an assembly of various ingredients (Figure I.1), the control of which is a real challenge for food technologists and engineers.

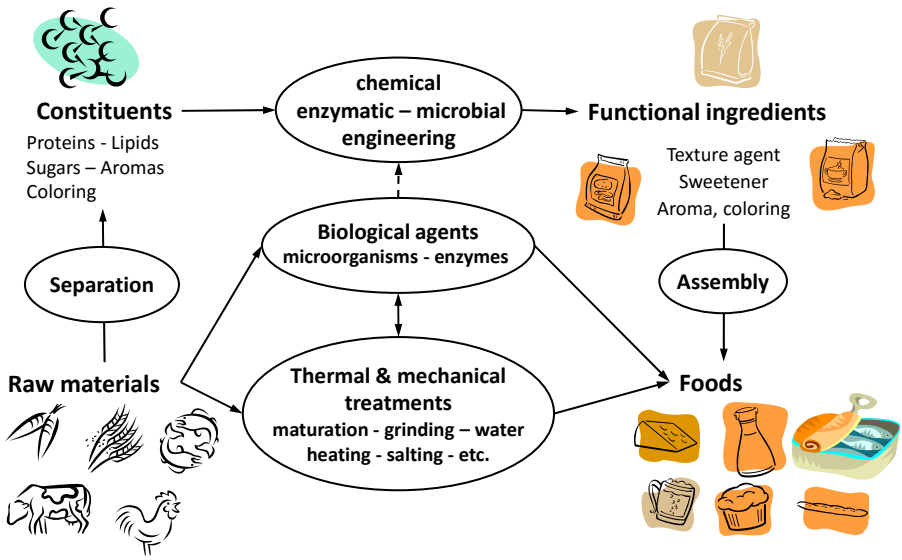

Figure I.1. *New products and assembly technology*

I.1. From empiricism to rational technology

The oldest forms of processing (milk to cheese, grain to bread or beer, grapes to wine, muscle to meat, etc.) were based on biological phenomena that could occur naturally under specific water content and temperature conditions, since the biological agents (enzymes or microorganisms) responsible for processing and the reaction substrates and growth factors were present in the raw materials and/or immediate environment; it was enough to simply mature (milk, meat), crush, grind and sometimes hydrate (fruit, grain) in order for biological reactions to take place. This is why these types of products were able to develop based solely on the observation of natural processes.

The knowledge acquired since the end of the 19th Century in the field of microbiology and the early 20th Century in the field of enzymology has gradually helped explain the biological phenomena involved in the development of certain food products. Based on this knowledge, the food industry has sought to control these processes rather than witness them,

which is how the fermentation and then an enzyme industry arose, producing and marketing biological agents for each type of processing. The use of fermentation and in some cases enzymes has become indispensable given the existing food safety requirements, which include increasingly stringent hygiene conditions in production and processing, and technological treatments to eliminate potential pathogenic microorganisms (microfiltration, heat treatment). This change has led to a reduction in the endogenous biological potential, which needs to be replenished by adding fermentation steps and enzymes. Reconstituting microbial ecosystems through the assembly of exogenous flora requires the identification of the endogenous flora and their role in the characteristic features of the food; progress in the field of molecular biology should enable significant progress in this area.

Over the past 40 years, many teams have focused on the study of food science, which has resulted in a better understanding of the composition of various raw materials and the biological and physicochemical mechanisms involved in the development of texture, flavor and aroma; this work has allowed the food industry to better identify the key technological tools in the development of quality, and to rely less on empiricism and more on technology.

I.2. From traditional foods to assembly technology

The quality requirements of consumers are increasingly specific and segmented. Food must be absolutely safe (no pathogens, toxins, residues or contaminants), have the closest possible nutritional profile to that recommended by nutritionists, meet sensory needs, integrate practicality (ease of storage and use) and convey social values (fair trade, environmental protection and animal welfare) while remaining at an affordable price. These market expectations are identified by the marketing services of the food industry for specific target consumers whose needs depend on several factors (gender, age, activity, health, metabolic disorders, food trends, etc.). These services, in consultation with nutrition and health specialists, identify which nutrients and micronutrients (minerals and vitamins) to assemble and define the structure, sensory characteristics and practicality of the food based on consumer research. The path from conception to completion can sometimes be difficult, since food is a complex and thermodynamically-unstable system, which can be defined as a continuous, usually aqueous phase, a three-dimensional protein and/or polysaccharide network and dispersed

elements (gas, fat globules, solids); the aim of technologists is to stabilize this system throughout the marketing period while taking into account mechanical (transport) and thermal (refrigeration, freezing, thawing) constraints.

The progress made in recent years in food science has provided insight into the key role of various biological components and particularly their structure, whether native or modified by technological treatments, in the development of texture, the thermodynamics of dispersed systems and the role of interfaces. This knowledge allows us to better understand and control the instability of food using technological processes or functional ingredients; the industry has a very large range of functional ingredients that is used to create texture and stabilize complex multiphase systems. It is therefore possible, by assembly, to create new foods that meet the quality requirements of the market.

PART 1

Food from Animal Sources

1

From Milk to Dairy Products

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));

Chapter written by Thomas CROGUENNEC, Romain JEANTET and Pierre SCHUCK.

Handbook of Food Science and Technology 3: *Food Biochemistry and Technology*,
First Edition. Edited by Romain Jeantet, Thomas Croguennec, Pierre Schuck and Gérard Brulé.

– 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).

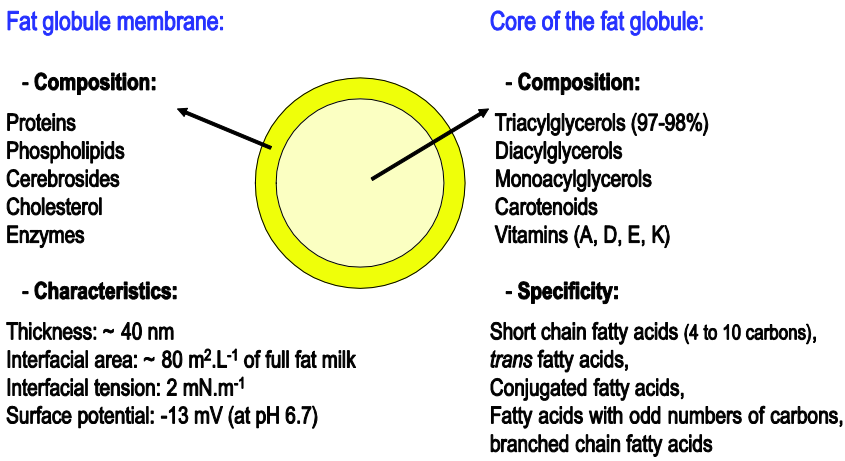

Figure 1.1. *Composition and main characteristics of milk fat globules*

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.

Class of lipids	*Percentage of total lipids (w/w)*
Triacylglycerols	97.5
Diacylglycerols	0.36
Monoacylglycerols	0.027
Free fatty acids	0.1
Cholesterol	0.31
Hydrocarbons	Traces
Carotenoids	0.008
Phospholipids	0.6

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.

Fatty acids	Symbol	% mol	Distribution on the glycerol sites (% mol)			Melting point (°C)
			Sn1	*Sn2*	*Sn3*	
Butyric	4:0	4.8	-	-	35.4	-7.9
Caproic	6:0	2.2	-	0.9	12.9	-1.5
Caprylic	8:0	1.3	1.4	0.7	3.6	+16.5
Capric	10:0	2.9	1.9	3.0	6.2	+31.4
Lauric	12:0	3.3	4.9	6.2	0.6	+43.6
Myristic	14:0	10.8	9.7	17.5	6.4	+53.8
Palmitic	16:0	26.1	34.0	32.3	5.4	+62.6
Palmitoleic	16:1	1.4	2.8	3.6	1.4	-0.5
Stearic	18:0	10.8	10.3	9.5	1.2	+69.3
Oleic	18:1	24.1	30.0	18.9	23.1	+14.0
Linoleic	18:2	2.4	1.7	2.5	2.3	-5.0
Linolenic	18:3	1.1	-	-	-	-11.0

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.

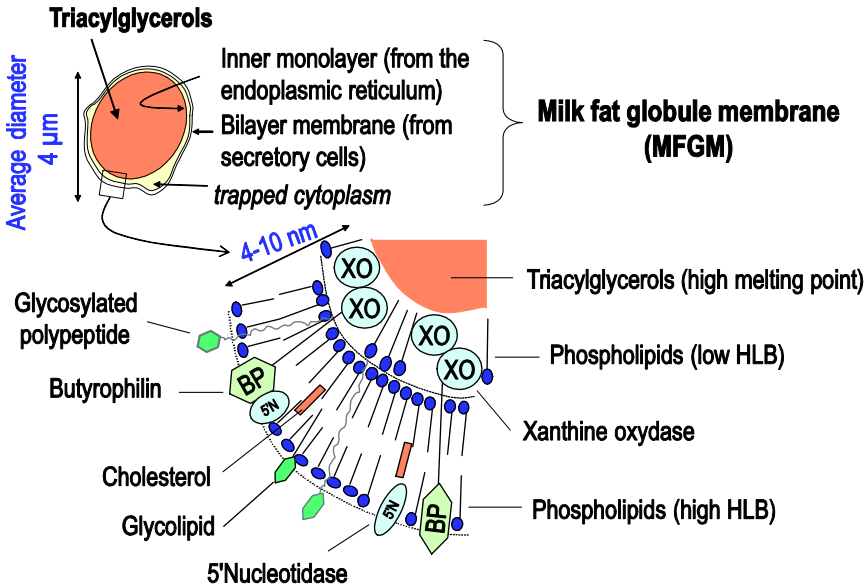

Figure 1.2. *Diagram of the structure of a native milk fat globule membrane (Source: [MIC 01]). For a color version of this figure, see www.iste.co.uk/jeantet/foodscience.zip*

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.

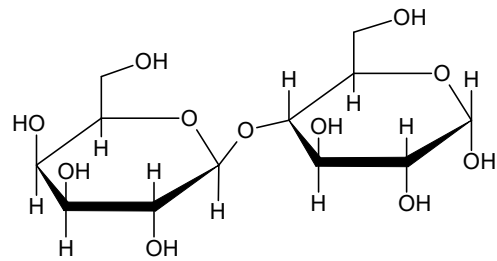

Figure 1.3. *Chemical structure of lactose*

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 (α_{S1}, α_{S2}, 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 amphiphilic 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.

Amino acid	*Caseins*			
	α_{S1} (B)	α_{S2} (A)	β (A)	κ
Asp	7	4	4	4
Glu	24	25	18	13
Asn	8	14	5	7
Gln	15	15	21	14
Thr	5	15	9	14
Ser	8	6	11	12
SerP	8	11	5	1
Pro	17	10	35	20
Gly	9	2	5	2
Ala	9	8	5	15
Val	11	14	19	11
Ile	11	11	10	13
Leu	17	13	22	8
Phe	8	6	9	4
Tyr	10	12	4	9
Met	5	4	6	2
Cys	0	0	0	0 or 2
Cystine/2	0	2	0	2 or 0
Lys	14	24	11	9
His	5	3	5	3
Arg	6	6	4	5
Trp	2	2	1	1
Total	199	207	209	169

Table 1.3. *Amino acid composition of cow's milk casein (α_{S1} (variant B), α_{S2} (variant A), β (variant A), casein κ)*

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. α_{s2}- and κ-caseins each have two cysteines involved in intermolecular disulphide bonds. While α_{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 (α_{S1}, α_{S2}, β and κ), some peptide fragments resulting from the proteolysis of β-casein by plasmin (γ-casein) and salt components, the main ones being calcium and phosphate. Table 1.4 shows the average composition of casein micelles.

Caseins		*Salt components*	
α_{S1}	33	Calcium	2.9
α_{S2}	11	Magnesium	0.2
β	33	Inorganic	4.3
κ	11	Citrate	0.5
γ	4		
Total caseins	92	Total salt components	8.0

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 α_{S1} and α_{S2} casein varies marginally, whereas the ratio of β- 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.

Property	*Values*
Average diameter (nm)	150
Area (cm^2)	8×10^{-10}
Volume (mL)	2.1×10^{-15}
Density (hydrated)	1.0632
Hydration (g H_2O g^{-1} of proteins)	3.7
Voluminosity (mL $g^{-1}$of proteins)	4.4
Molecular weight (hydrated) (Da)	1.3×10^9
Molecular weight (dehydrated) (Da)	5×10^8
Water content (%, w/w)	63
Number of caseins per micelle	2×10^4
Number of nanoclusters of calcium phosphate per micelle	3×10^3
Number of micelles per L of milk	$10^{17} – 10^{19}$
Mean free distance between micelles (nm)	240
Zeta potential (mV)	–13

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