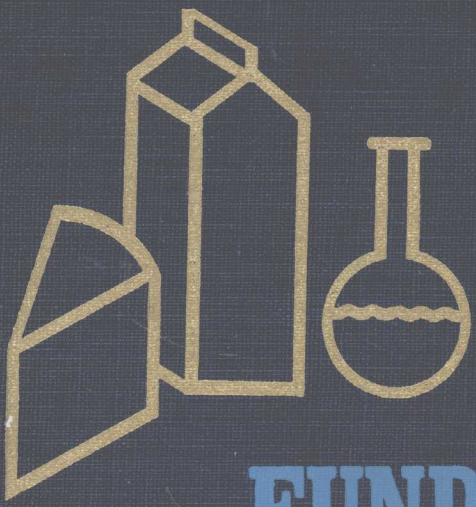

FUNDAMENTALS OF DAIRY CHEMISTRY

THIRD EDITION

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FUNDAMENTALS OF DAIRY CHEMISTRY

THIRD EDITION

To
BYRON H. WEBB

for his outstanding dedicated service to the dairy industry that spans half a century and whose persistence and guidance has led to another edition of Fundamentals.

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Preface

Fundamentals of Dairy Chemistry has always been a reference text which has attempted to provide a complete treatise on the chemistry of milk and the relevant research. The third edition carries on in that format which has proved successful over four previous editions (*Fundamentals of Dairy Science* 1928, 1935 and *Fundamentals of Dairy Chemistry* 1965, 1974). Not only is the material brought up-to-date, indeed several chapters have been completely re-written, but attempts have been made to streamline this edition. In view of the plethora of research related to dairy chemistry, authors were asked to reduce the number of references by eliminating the early, less significant ones. In addition, two chapters have been replaced with subjects which we felt deserved attention: "Nutritive Value of Dairy Foods" and "Chemistry of Processing." Since our society is now more attuned to the quality of the food it consumes and the processes necessary to preserve that quality, the addition of these topics seemed justified. This does not minimize the importance of the information in the deleted chapters, "Vitamins of Milk" and "Frozen Dairy Products." Some of the material in these previous chapters has been incorporated into the new chapters; furthermore, the information in these chapters is available in the second edition, as a reprint from ADSA (Vitamins in Milk and Milk Products, November 1965) or in the many texts on ice cream manufacture.

Originally, *Fundamentals of Dairy Science* (1928) was prepared by members of the Dairy Research Laboratories, USDA. Over the years, the trend has changed. The present edition draws heavily from the expertise of the faculty and staff of universities. Ten of the 14 chapters are written by authors from state universities, three from ARS, USDA, and one from industry.

It seems fitting that this is so. The bulk of future dairy research, if it is to be done, appears destined to be accomplished at our universities. Hopefully the chapter authors have presented appropriate material and in such a way that it serves best the principal users of this book, their students. As universities move away from specific product technology and food technology becomes more sophisticated, a void has

been created where formerly a dairy curriculum existed. It is hoped that this edition of *Fundamentals of Dairy Chemistry* which incorporates a good deal of technology with basic chemistry can help fill this void.

Preparation of this volume took considerably longer than anticipated. The exigencies of other commitments took its toll. Originally the literature was supposed to be covered to 1982 but many of the chapters have more recent references.

I wish to acknowledge with appreciation the contribution made by the chapter authors and the associate editors. Obviously without their assistance, publication of this edition would not have been possible. Dr. Jenness was responsible for Chapters 1, 3, 8, and 9; Dr. Keeney, Chapters 4, 5, and 10; Dr. Marth, Chapters 2, 13, and 14; and Dr. Wong, Chapters 6, 7, 11 and 12.

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1

Composition of Milk

Robert Jenness

Milk is secreted by all species of mammals to supply nutrition and immunological protection to the young. It performs these functions with a large array of distinctive compounds. Interspecies differences in the quantitative composition of milk (Jenness and Sloan 1970) probably reflect differences in the metabolic processes of the lactating mother and in the nutritive requirements of the suckling young.

Human beings consume large amounts of milk of a few species besides their own. The principal ones are cows, water buffaloes, goats, and sheep, which furnish annually about 419, 26, 7.2, and 7.3 million metric tons of milk, respectively, for human consumption *(FAO Production Yearbook* 1979). This chapter, and indeed this entire volume, deals primarily with the milk of western cattle—*Bos taurus.* References to reviews concerning milk of other important species are: Indian cattle—*B. indicus* (Basu *et al.* 1962); water buffalo—*Bubalus bubalis* (Laxminarayan and Dastur 1968); goat—*Capra hircus* (Parkash and Jenness 1968; Jenness 1980; Ramos and Juarez 1981); sheep—*Ovis aries* (Ramos and Juarez 1981); and humans—*Homo sapiens* (Macy *et al.* 1953; Jenness 1979; Blanc 1981; Gaull *et al.* 1982; Packard 1982).

In the United States, milk is defined for commercial purposes as the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows, which contains not less than 8.25% of milk-solids-not-fat and not less than 3.25% milk fat. Minimal standards in the various states may vary from 8.0 to 8.5% for milk-solids-not-fat and from 3.0 to 3.8% for milk fat (U.S. Dept. Agr. 1980).

CONSTITUENTS OF MILK

Milk consists of water, lipids, carbohydrates, proteins, salts, and a long list of miscellaneous constituents. It may contain as many as 10^5 different kinds of molecules. Refinement of qualitative and quantitative techniques continues to add new molecular species to the list. The constituents fall into four categories:

1. Organ and species specific—most proteins and lipids.
2. Organ but not species specific—lactose.
3. Species but not organ specific—some proteins.
4. Neither organ nor species specific—water, salts, vitamins.

The following sections summarize the constituents of milk and indicate how they are quantitated operationally. Detailed descriptions and properties of lipids, lactose, and proteins will be found in later chapters.

Water

Milks of most species contain more water than any other constituent. Certainly this is true of the milks consumed by humans. The other constituents are dissolved, colloidally dispersed, and emulsified in water. The dissolved solutes in bovine milk aggregate about 0.3 M and depress the freezing point by about 0.54°C (see Chapter 8). The activity of water in milk, a_w, which is the ratio of its vapor pressure to that of air saturated with water, is about 0.993. A small amount of the water of milk is "bound" so tightly by proteins and by the fat globule membrane that it does not function as a solvent for small molecules and ions. Water content is usually determined as loss in weight upon drying under conditions that minimize decomposition of organic constituents, e.g., 3 hr at 98–100°C (Horwitz 1980).

Lipids

The lipids of milk, often simply called "fat," consist of materials that are extractable by defined methods. Simple extraction with a nonpolar solvent like ether or chloroform is not efficient because the fat is located in globules protected by a surface membrane. A widely used gravimetric method is the Roese-Gottlieb extraction (Walstra and Mulder 1964) using NH_4OH, ethanol, diethyl ether, and petroleum ether. Volumetric methods such as that of Babcock and Gerber (Ling 1956; Horwitz 1980) use H_2SO_4 to liberate the fat, which is then measured. Rapid determination of the amount of fat in milk can be done by measurement of the absorption of infrared radiation at 3.4 or 5.7 μm (Chapter 8; Goulden 1964; Horwitz 1980).

The lipids of milk are composed of about 98% triglycerides, with much smaller amounts of free fatty acids, mono-and diglycerides, phospholipids, sterols, and hydrocarbons. Chapter 4 deals in detail with the composition of milk lipids.

The fat in milk is almost entirely in the form of globules, ranging

from 0.1 to 15 μm in diameter. Size distribution is an inherited characteristic that varies among species and among breeds of cattle. Bovine milk contains many very small globules that comprise only a small fraction of the total fat. The total number is about 15×10^9 globules per milliliter of which 75% are smaller than 1 μm in diameter. The fat globules and their protective membrane of phospholipids and proteins are described in Chapter 10.

Carbohydrates

In bovine milk, and indeed in all milks consumed by humans, the overwhelming carbohydrate is lactose. This disaccharide, 4-0-β-D-galactopyranosyl-D-glucopyranose, is a distinctive and unique product of the mammary gland. It has been found in milks of almost all of the species analyzed to date (Jenness *et al.* 1964) and nowhere else in nature except in low concentration in the fruits of some of the Sapotaceae (Reithel and Venkataraman 1956). Lactose is discussed in detail in Chapter 6.

Lactose in milk can be quantitated by oxidation of the aldehyde of the glucose moiety (Hinton and Macara 1927; McDowell 1941; Perry and Doan 1950; Horwitz 1980), by polarimetry of a clarified solution (Grimbleby 1956; Horwitz 1980), by colorimetry of the product of reaction with phenolic compounds (Marier and Boulet 1959), by infrared absorption at 9.6 μm (Goulden 1964; Horwitz 1980), by enzymatic assay with β-galactosidase and galactose dehydrogenase (Kurz and Wallenfels 1974), and by chromatography (Reineccius *et al.* 1970; Beebe and Gilpin 1983; Brons and Olieman 1983). Only the last two of these methods are specific for lactose, but bovine milk contains so little other material that is oxidizable, that exhibits optical rotation, that reacts with phenolic compounds, or that absorbs at 9.6 μm that the first four give reasonable estimates of lactose. Older analyses were made by oxidation or polarimetry. Published values for lactose contents obtained with these methods must be scrutinized carefully because some were calculated on the basis of lactose monohydrate and are thus 5.26% too high (360/342).

Carbohydrates other than lactose in milk include monosaccharides, neutral and acid oligosaccharides, and glycosyl groups bound to proteins and lipids. Glucose and galactose are detectable by thin layer chromatography (TLC) and gas-liquid chromatography (GLC) of bovine milk. Of course, hydrolysis of lactose is an obvious source of these two monosaccharides, but with precautions taken to avoid hydrolysis, concentrations of 100–150 mg/liter of each have been found by GLC (Reineccius *et al.* 1970). Specific enzymatic methods, however, have indicated considerably lower concentrations—about 30 mg glucose and

90 mg galactose per liter (Faulkner *et al.* 1981). Free *myo*-inositol has been found in the milks of several species (in addition to that bound in phosphatidyl inositols; (see Chapter 4). Bovine milk has only 40–50 mg of *myo*-inositol per liter, but milks of some other species contain much more (Byun and Jenness 1982).

The carbohydrates L(-)fucose (Fuc), N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose), N-acetyl galactosamine (2-acetamido-2-deoxy-D-galactose), and N-acetylneuraminic acid occur in milk almost entirely in the form of oligosaccharides and glycopeptides. Only small concentrations are present in the free state, although there is one report of 112 mg of N-acetylglucosamine per liter of bovine milk (Hoff 1963). The total (free and combined) content of N-acetylneuraminic acid is 100–300 mg/liter (de Koning and Wijnand 1965).

Bovine milk contains 1–2 g of oligosaccharides per liter, human milk 10–25 g/liter. Colostrums of both species have higher concentrations. A recent review (Blanc 1981) lists more than 60 oligosaccharides which have been detected in human milk. They range from 3 to 20 monosaccharide units per molecule; not all have been characterized structurally. Five oligosaccharides have been detected and characterized in bovine milk or colostrum. All of the oligosaccharides that have been characterized in either bovine or human milk have a lactose moiety, D-Gal-β-(1-4)-D-Glc in the reducing terminal position. (In a few, the terminal residue is an N-acetylglucosamine). The simplest are the trisaccharides fucosyllactose [L-Fuc α-(1–2)-D-Gal-β-(1–4)-D-Glc] and N-acetylneuraminyllactose [NANA-(2–3)-D-Gal-β-(1–4)-D-Glc]. More complex ones have longer chains with various kinds of branching (Ebner and Schanbacher 1974; Blanc 1981).

Various sugar phosphates occurring in milk are listed later under miscellaneous constituents. The glycosyl groups of several of the milk proteins are described in Chapter 3.

Proteins

The proteins of milk fall into several classes of polypeptide chains. These have been delineated most completely in bovine milk, and a system of nonmenclature has been developed for them (Chapter 3; Eigel *et al.* 1984). One group, called "caseins," consists of four kinds of polypeptides: α_{s1}-, α_{s2}-, and β-, and κ- with some genetic variants, post translational modifications, and products of proteolysis. Almost all of the caseins are associated with calcium and phosphate in micelles 20–300 μm in diameter (see Chapter 9). The other milk proteins, called "whey proteins," are a diverse group including β-lactoglobulin, α-lactalbumin, blood serum albumin, and immunoglobulins (Chapter 3). Almost all

milk proteins of nonbovine species defined to date appear to be evolutionary homologs of those of the bovine and are named accordingly.

Classically, milk protein content has been determined by Kjeldahl analysis for nitrogen (N) (Horwitz 1980). This has the advantages that N is a major constituent, comprising about one-sixth of the mass of the protein, and that the N contents of the individual milk proteins are nearly the same. Multiplication by 6.38 has been used commonly to convert the N content to protein. This is based on an old determination of 15.67% N in milk proteins, but a modern weighted average of the N contents of individual milk proteins indicates that the factor should be 6.32 (Walstra and Jenness 1984). Thus older results may be nearly 1% too high. A more serious error is that protein contents have often been calculated as 6.38 $\times$ total N. Such "crude protein" values are 4–8% too high because they include N from nonprotein nitrogenous constituents.

Various procedures are used to separate milk proteins into fractions or individual components that can quantitated separately. A classic method of fractionation is by precipitation at pH 4.6, which separates the proteins into two groups—caseins in the precipitate and whey proteins in the supernatant. All proteins are precipitated from a second aliquot with trichloroacetic acid at 12% (w/v) concentration (Rowland 1938), and concentrations of casein and whey proteins are calculated as follows:

$$\text{Casein} = 6.38\ (\text{TN-NCN})$$
$$\text{Whey protein} = 6.38\ (\text{NCN-NPN})$$

where TN is total nitrogen, NCN is nitrogen in the pH 4.6 filtrate, and NPN is nitrogen in the trichloroacetic acid filtrate. About 80% of the proteins of bovine milk fall into the category of caseins, but the proportions differ greatly among species (see Table 1.10).

Numerous other methods have been proposed for routine determination of protein on large numbers of samples. Several are reviewed by Booy *et al.* (1962). They include colorimetric determination of ammonia, colorimetric determination of peptide linkages by the biuret method, analysis for tyrosyl groups, titration of protons released from lysyl groups upon reaction with formaldehyde, binding of anionic dyes to cationic protein groups, turbidimetric procedures (Kuramoto *et al.* 1959), and absorption of infrared radiation of 6.46 μm (Goulden 1964; Horwitz 1980). Individual milk proteins can be assayed by specific immunological tests (Larson and Twarog 1961; Larson and Hageman 1963; Babajimopoulos and Mikolajcik 1977; Guidry and Pearson 1979; Devery-Pocius and Larson 1983), by ion-exchange chromatography (Davies and Law 1977), by gel filtration (Davies 1974), by zone electro-

phoresis (Swaisgood 1975; West and Towers 1976, Bell and Stone 1979), and by high performance liquid chromatography (Diosady *et al.* 1980; Bican and Blanc 1982).

Salts

For the purpose of this discussion, milk salts are considered as ionized or ionizable substances of molecular weight 300 or less. Ionizable groups of proteins are not included here, although, of course, they must be taken into account in a complete description of ionic balance and equilibria. Trace elements, some of which are ionized or partially so in milk, are considered in a later section of this chapter. Milk salts include both inorganic and organic substances; thus they are not equivalent to either minerals or ash. The principal cations are Na, K, Ca, and Mg, and the anionic constituents are phosphate, citrate, chloride, carbonate, and sulfate. Small amounts of amino cations and organic acid anions are also present.

General methods for quantitating minerals (especially metals) use absorption or emission of radiation of specific wavelengths (Wenner 1958; Murthy and Rhea 1967). The former is a measure of absorption of the energy required to raise electrons to a higher energy-excited state and the latter is a measure of the energy released when excited electrons revert to their original state. These methods are particularly suitable for Na and K, for neither of which are volumetric or gravimetric methods of sufficient sensitivity available. Calcium and magnesium can also be determined by emission or absorption but often are analyzed by specific chemical methods. Dry ashing or wet digestion with H_2SO_4-H_2O_2 or HNO_3–$HClO_4$ are often used to destroy organic material before analysis for minerals, but in some procedures diluted, unfractionated samples are injected directly into the flame photometer. Defatted and deproteinized extracts, usually acid, are used to determine the content of organic salts such as citrate; they are sometimes used for analyses of mineral constituents as well.

Classically, calcium was determined by precipitation as calcium oxalate, which was then titrated in H_2SO_4 solution with $KMnO_4$ but this has been largely replaced by titration with the chelating agent ethylenediamine tetraacetate (EDTA), using as the indicator a dye (murexide) which changes color when it binds calcium (White and Davies 1962). Another more sensitive method for Ca determination is a colorimetric procedure using glyoxal *bis* (2-hydroxyanil), whose calcium complex absorbs strongly at 524 nm (Nickerson *et al.* 1964). Phosphate interferes with both methods; it can be removed by treatment with an anion exchanger or by precipitation with potassium *meta*-stannate. Alterna-

tively, the calcium can be precipitated as oxalate before titration with EDTA.

Magnesium, formerly determined by precipitation as magnesium ammonium phosphate and determining P in the latter, can be analyzed readily by EDTA titrations. It can be obtained either as the difference between titrations for (Ca and Mg) and Ca alone or by titrating the supernatant after Ca is precipitated as oxalate (White and Davies 1962).

Phosphate is determined almost universally by its reaction with molybdate to form phosphomolybdate. The latter can be reduced to a blue compound that absorbs at various wavelengths, of which 640 and 820 nm are often used for colorimetric quantitation (Allen 1940; Sumner 1944; Meun and Smith, 1968).

Chloride is analyzed by some form of reaction with silver to form insoluble silver chloride. Direct titration of milk with silver nitrate yields erroneously high and variable results, and pre-ashing cannot be used because chloride is lost by volatilization. Satisfactory procedures involve adding an excess of standardized $AgNO_3$ directly to milk and back titrating with potassium thiocyanate (KSCN), using a soluble ferric salt as the indicator (Sanders 1939).

Citrate may be oxidized with $KMnO_4$ and brominated and decarboxylated to form the relatively insoluble pentabromacetone; certain methods for detecting citrate in milk, including that of the Association of Official Analytical Chemists (Horwitz 1980), employ this reaction for a gravimetric analysis. It is, however, cumbersome, and pentabromacetone is somewhat more soluble and volatile than desired in a gravimetric analysis. In another method, lead citrate is precipitated from a sulfuric acid–alcohol filtrate from milk and titrated with ammonium perchlorato-cerate (Heinemann 1944). A simpler and more sensitive procedure utilizes the Furth-Herrmann reaction, in which a yellow-colored condensation product of citrate with pyridine is formed in the presence of acetic anhydride (White and Davies 1963). Citrate may also be determined enzymatically by cleavage with a bacterial citrate lyase to oxaloacetate; decarboxylation of the latter to pyruvate with oxaloacetate decarboxylase; and finally, formation of malate and lactate with specific NAD-coupled dehydrogenases (Dagley 1974). The enzymatic method is the most specific method yet employed. About 10% of the total apparent citrate of milk actually is isocitrate (Faulkner and Clapperton 1981).

Inorganic sulfate, SO_4^{2-}, is present in milk in a concentration of about 1mM; it may be determined turbidimetrically after adding barium ion to a deproteinized filtrate (Koops 1965).

The total carbonate system (mostly HCO_3^- in equilibrium with CO_2)