11   Physical properties of milk

Milk is a dilute emulsion consisting of an oil/fat dispersed phase and an aqueous colloidal continuous phase. The physical properties of milk are similar to those of water but are modified by the presence of various solutes (proteins, lactose and salts) in the continuous phase and by the degree of dispersion of the emulsified and colloidal components.

Data on the physical properties of milk are important since such parameters can influence the design and operation of dairy processing equipment (e.g. thermal conductivity or viscosity) or can be used to determine the concentration of specific components in milk (e.g. use of the elevation in freezing point to estimate added water or specific gravity to estimate solids-not-fat), or to assess the extent of biochemical changes in the milk during processing (e.g. acidification by starter or the development of a rennet coagulum). Some important physical properties of milk are summarized in Table 11.1.

Table 11.1 Some physical properties of milk (Walstra and Jenness, 1984; Sherbon, 1988; Singh, McCarthy and Lucey, 1997)

Osmotic pressure	~700 kPa
a_w	~0.993
Boiling point	~100.15°C
Freezing point	−0.522°C (approx.)
Refractive index, n_D^{20}	1.3440–1.3485
Specific refractive index	~0.2075
Density (20°C)	~1030 kg m^{-3}
Specific gravity (20°C)	~1.0321
Specific conductance	~0.0050 ohm^{-1} cm^{-1}
Ionic strength	~0.08 M
Surface tension (20°C)	~52 N m^{-1}
Coefficient of viscosity	2.127 mPa s
Thermal conductivity (2.9% fat)	~0.559 W m^{-1} K^{-1}
Thermal diffusivity (15–20°C)	~1.25 × 10^{-7} m^2 s^{-1}
Specific heat	~3.931 kJ kg^{-1} K^{-1}
pH (at 25°C)	~6.6
Titratable acidity	1.3–2.0 meq OH$^-$ per 100 ml (0.14–0.16% as lactic acid)
Coefficient of cubic expansion (273–333 K)	0.0008 m^3 m^{-3} K^{-1}
Redox potential (25°C, pH 6.6, in equilibrium with air)	+0.25 to +0.35 V

11.1 Ionic strength

The ionic strength, I, of a solution is defined as:

$$I = \tfrac{1}{2}\Sigma c_i z_i^2 \tag{11.1}$$

where c_i is the molar concentration of the ion of type i and z_i is its charge. The ionic strength of milk is c. 0.08 M.

11.2 Density

The density (ρ) of a substance is its mass per unit volume, while its specific gravity (SG) or relative density is the ratio of the density of the substance to that of water (ρ_w) at a specified temperature:

$$\rho = m/V \tag{11.2}$$

$$SG = \rho/\rho_w \tag{11.3}$$

$$\rho = SG\rho_w \tag{11.4}$$

The thermal expansion coefficient governs the influence of temperature on density and therefore it is necessary to specify temperature when discussing density or specific gravity. The density of milk is of consequence since fluid milk is normally retailed by volume rather than by mass. Measurement of the density of milk using a hydrometer (lactometer) has also been used to estimate its total solids content.

The density of bulk milk (4% fat and 8.95% solids-not-fat) at 20°C is approximately $1030\,\mathrm{kg\,m^{-3}}$ and its specific gravity is 1.0321. Milk fat has a density of about $902\,\mathrm{kg\,m^{-3}}$ at 40°C. The density of a given milk sample is influenced by its storage history since it is somewhat dependent on the liquid to solid fat ratio and the degree of hydration of proteins. To minimize effects of thermal history on its density, milk is usually prewarmed to 40–45°C to liquify the milk fat and then cooled to the assay temperature (often 20°C).

The density and specific gravity of milk vary somewhat with breed. Milk from Ayrshire cows has a mean specific gravity of 1.0317 while that of Jersey and Holstein milks is 1.0330. Density varies with the composition of the milk and its measurement has been used to estimate the total solids content of milk. The density of a multicomponent mixture (like milk) is related to the density of its components by:

$$1/\rho = \Sigma(m_x/\rho_x) \tag{11.5}$$

where m_x is the mass fraction of component x, and ρ_x its apparent density in the mixture. This apparent density is not normally the same as the true density of the substance since a contraction usually occurs when two components are mixed.

Equations have been developed to estimate the total solids content of milk based on % fat and specific gravity (usually estimated using a lactometer). Such equations are empirical and suffer from a number of drawbacks; for further discussion see Jenness and Patton (1959). The principal problem is the fact that the coefficient of expansion of milk fat is high and it contracts slowly on cooling and therefore the density of milk fat (Chapter 3) is not constant. Variations in the composition of milk fat and in the proportions of other milk constitiuents have less influence on these equations than the physical state of the fat.

In addition to lactometry (determination of the extent to which a hydrometer sinks), the density of milk can be measured by pycnometry (determination of the mass of a given volume of milk), by hydrostatic weighing of an immersed bulb (e.g. Westphal balance), by dialatometry (measurement of the volume of a known mass of milk) or by measuring the distance that a drop of milk falls through a density gradient column.

11.3 Redox properties of milk

Oxidation–reduction (redox) reactions involve the transfer of an electron from an electron donor (reducing agent) to an electron acceptor (oxidizing agent). The species that loses electrons is said to be oxidized while that which accepts electrons is reduced. Since there can be no net transfer of electrons to or from a system, redox reactions must be coupled and the oxidation reaction occurs simultaneously with a reduction reaction.

The tendency of a system to accept or donate electrons is measured using an inert electrode (typically platinum). Electrons can pass from the system into this electrode, which is thus a half-cell. The Pt electrode is connected via a potentiomenter to another half-cell of known potential (usually, a saturated calomel electrode). All potentials are referred to the hydrogen half-cell:

$$\tfrac{1}{2}H_2 \rightleftarrows H^+ + e^- \tag{11.6}$$

which by convention is assigned a potential of zero when an inert electrode is placed in a solution of unit activity with respect to H^+ (i.e. pH = 0) in equilibrium with H_2 gas at a pressure of 1.013×10^5 Pa (1 atm). The redox potential of a solution, E_h, is the potential of the half-cell at the inert electrode and is expressed as volts. E_h depends not only on the substances present in the half-cell but also on the concentrations of their oxidized and reduced forms. The relationship between E_h and the concentrations of the oxidized and reduced forms of the compound is described by the Nernst equation:

$$E_h = E_o - RT/nF \ln a_{red}/a_{ox} \tag{11.7}$$

where E_o is the standard redox potential (i.e. potential when reactant and

product are at unit activity), n is the number of electrons transferred per molecule, R is the universal gas constant $(8.314 \, J \, K^{-1} \, mol^{-1})$, T is temperature (in Kelvin), F is the Faraday constant $(96.5 \, kJ \, V^{-1} \, mol^{-1})$ and a_{red} and a_{ox} are activities of the reduced and oxidized forms, respectively. For dilute solutions, it is normal to approximate activity by molar concentration. Equation 11.7 can be simplified, assuming a temperature of 25°C, a transfer of one electron and that activity $\approx$ concentration:

$$E_h = E_o + 0.059 \log [Ox]/[Red]. \tag{11.8}$$

Thus, E_h becomes more positive as the concentration of the oxidized form of the compound increases. E_h is influenced by pH since pH affects the standard potential of a number of half-cells. The above equation becomes:

$$E_h = E_o + 0.059 \log [Ox]/[Red] - 0.059 \, pH. \tag{11.9}$$

The E_h of milk is usually in the range $+0.25$ to $+0.35$ V at 25°C, at pH 6.6 to 6.7 and in equilibrium with air (Singh, McCarthy and Lucey, 1997). The influence of pH on the redox potential of a number of systems is shown in Figure 11.1.

The concentration of dissolved oxygen is the principal factor affecting the redox potential of milk. Milk is essentially free of O_2 when secreted but in equilibrium with air, its O_2 content is about $0.3 \, mM$. The redox potential of anaerobically drawn milk or milk which has been depleted of dissolved oxygen by microbial growth or by displacement of O_2 by other gases is more negative than that of milk containing dissolved O_2.

The concentration of ascorbic acid in milk $(11.2-17.2 \, mg \, l^{-1})$ is sufficient to influence its redox potential. In freshly drawn milk, all ascorbic acid is in the reduced form but can be oxidized reversibly to dehydroascorbate, which is present as a hydrated hemiketal in aqueous systems. Hydrolysis of the lactone ring of dehydroascorbate, which results in the formation of 2,3-diketogulonic acid, is irreversible (Figure 11.2).

The oxidation of ascorbate to dehydroascorbate is influenced by O_2 partial pressure, pH and temperature and is catalysed by metal ions (particularly Cu^{2+}, but also Fe^{3+}). The ascorbate/dehydroascorbate system in milk stabilizes the redox potential of oxygen-free milk at $c.$ 0.0 V and that of oxygen-containing milk at $+0.20$ to $+0.30$ V (Sherbon, 1988). Riboflavin can also be oxidized reversibly but its concentration in milk ($c.$ $4 \, \mu M$) is thought to be too low to have a significant influence on redox potenial. The lactate–pyruvate system (which is not reversible unless enzyme-catalysed) is thought not to be significant in influencing the redox potential of milk since it, too, is present at very low concentations. At the concentrations at which they occur in milk, low molecular mass thiols (e.g. free cysteine) have an insignificant influence on the redox potential of milk. Thiol–disulphide interactions between cysteine residues of proteins influence the redox properties of heated milks in which the proteins are denatured. The free
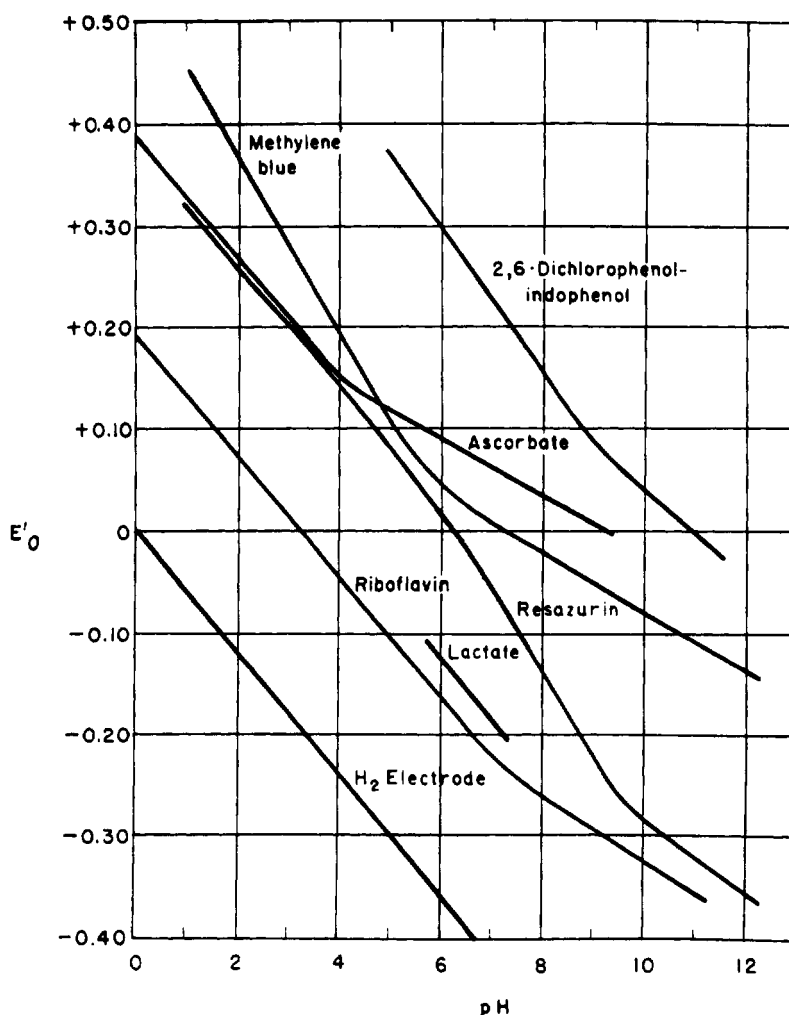
Figure 11.1 Effect of pH on the oxidation–reduction potential of various systems (from Sherbon, 1988).

aldehyde group of lactose can be oxidized to a carboxylic acid (lactobionic acid) at alkaline pH but this system contributes little to the redox properties of milk at pH 6.6.

The E_h of milk is influenced by exposure to light and by a number of processing operations, including those which cause changes in the concentration of O_2 in the milk. Addition of metal ions (particularly Cu^{2+}) also influences the redox potential. Heating of milk causes a decrease in its E_h,
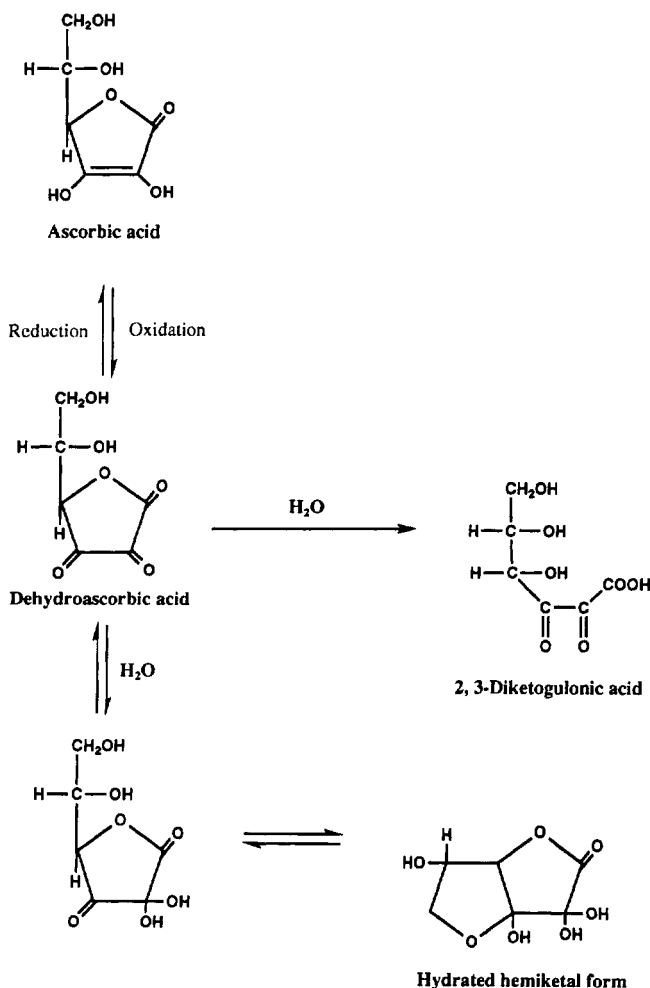
Figure 11.2 Chemical structures of ascorbic acid and its derivatives.

due mainly to the denaturation of β-lactoglobulin (and the consequent exposure of —SH groups) and loss of O_2. Compounds formed by the Maillard reaction between lactose and proteins can also influence the E_h of heated milk, particularly dried milk products.

Fermentation of lactose during the growth of micro-organisms in milk has a major effect on its redox potential. The decrease in the E_h of milk caused by the growth of lactic acid bacteria is shown in Figure 11.3. A rapid decrease in E_h occurs after the available O_2 has been consumed by the bacteria. Therefore, the redox potential of cheese and fermented milk products is negative. Reduction of redox indicators (e.g. resazurin or
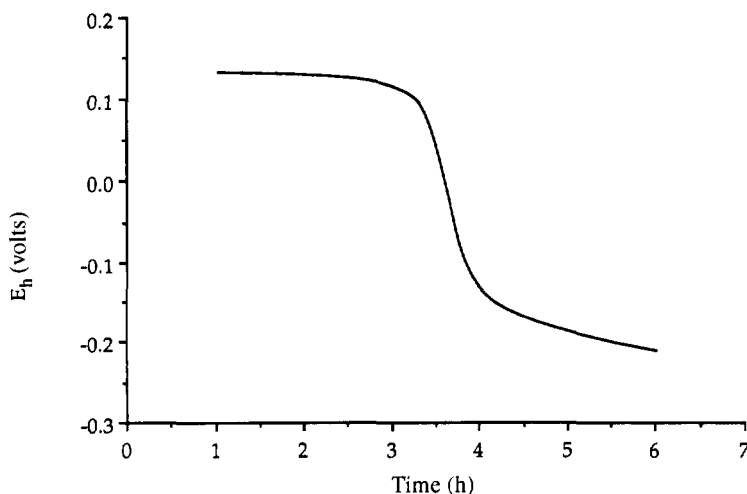
Figure 11.3 Decrease in the redox potential of milk caused by the growth of *Lactococcus lactis* subsp. *lactis at* 25°C.

methylene blue) can be used as an index of the bacterial quality of milk by measuring the 'reduction time', at a suitable temperature, of milk containing the dye.

Riboflavin absorbs light maximally at about 450 nm and in doing so can be excited to a triplet state. This excited form of riboflavin can interact with triplet O_2 to form a superoxide anion O_2^- (or H_2O_2 at low pH). Excited riboflavin can also oxidize ascorbate, a number of amino acids and proteins and orotic acid. Riboflavin-catalysed photo-oxidation results in the production of a number of compounds, most notably methional (**11.1**) which is the principal compound responsible for the off-flavour in milk exposed to light.
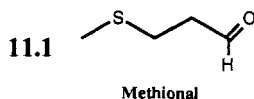
11.1

Methional

Photo-oxidation of milk constituents was discussed in detail by Walstra and Jenness (1984).

11.4 Colligative properties of milk

Colligative properties are those physical properties which are governed by the number, rather than the kind, of particles present in solution. The important colligative properties of milk are its freezing and boiling points (*c.* -0.522 and $100.15°C$, respectively) and its osmotic pressure (approxi-

mately 700 kPa at 20°C), all of which are interrelated. Since the osmotic pressure of milk remains essentially constant (because it is regulated by that of the cow's blood), the freezing point is also relatively constant.

The freezing point of an aqueous solution is governed by the concentration of solutes in the solution. The relationship between the freezing point of a simple aqueous solution and concentration of solute is described by a relation based on Raoult's law:

$$T_f = K_f m \qquad (11.10)$$

where T_f is the difference between the freezing point of the solution and that of the solvent, K_f is the molal depression constant (1.86°C for water) and m is the molal concentration of solute. However, this equation is valid only for dilute solutions containing undissociated solutes. Raoult's law is thus limited to approximating the freezing point of milk.

The freezing point of bovine milk is usually in the range -0.512 to -0.550°C, with a mean value close to -0.522°C (Sherbon, 1988) or -0.540°C (Jenness and Patton, 1959). Despite variations in the concentrations of individual solutes, the freezing point depression of milk is quite constant since it is proportional to the osmotic pressure of milk (approximately 700 kPa at 20°C), which is regulated by that of the cow's blood. The freezing point of milk is more closely related to the osmotic pressure of mammary venous blood than to that of blood from the jugular vein.

Owing to their large particle or molecular mass, fat globules, casein micelles and whey proteins do not have a significant effect on the freezing point of milk, to which lactose makes the greatest contribution. The freezing point depression in milk due to lactose alone has been calculated to be 0.296°C. Assuming a mean freezing point depression of 0.522°C, all other constituents in milk depress the freezing point by only 0.226°C. Chloride is also an important contributor to the colligative properties of milk. Assuming a Cl^- concentration of 0.032 M and that Cl^- is accompanied by a monovalent cation (i.e. Na^+ or K^+), the freezing point depression caused by Cl^- and its associated cation is 0.119°C. Therefore, lactose, chloride and its accompanying cations together account for about 80% of the freezing point depression in milk. Since the total osmotic pressure of milk is regulated by that of the cow's blood, there is a strong inverse correlation between lactose and chloride concentrations (Chapter 5).

Natural variation in the osmotic pressure of milk (and hence freezing point) is limited by the physiology of the mammary gland. Variations in the freezing point of milk have been attributed to seasonality, feed, stage of lactation, water intake, breed of cow, heat stress and time of day. These factors are often interrelated but have relatively little influence on the freezing point of milk. Likewise, unit operations in dairy processing which do not influence the net number of osmotically active molecules/ions in solution do not influence the freezing point. Cooling or heating milk causes

transfer of salts to or from the colloidal state. However, evidence for an effect of cooling or moderate heating (e.g. HTST pasteurization or minimum UHT processing) on the freezing point of milk is contradictory, perhaps since such changes are slowly reversible over time. Direct UHT treatment involves the addition of water (through condensed steam). This additional water should be removed during flash cooling, which also removes gases, e.g. CO_2, from the milk, causing a small increase in freezing point. Vacuum treatment of milk, i.e. vacreation (to remove taints), has been shown to increase its freezing point, presumably by degassing. However, if vacuum treatment is severe enough to cause a significant loss of water, the freezing point will be reduced, thus compensating fully or partially for the loss of CO_2. Fermentation of milk has a large effect on its freezing point since fermentation of 1 mol lactose results in the formation of 4 mol lactic acid. Likewise, fermentation of citrate influences the freezing point of milk.

Accurate measurement of the freezing point depression in milk requires great care. The principle used is to supercool the milk sample (by 1.0 to 1.2°C), to induce crystallization of ice, after which the temperature increases rapidly to the freezing point of the sample (Figure 11.4). For water, the temperature at the freezing point will remain constant until all the latent heat of fusion has been removed (i.e. until all the water is frozen). However, for milk the temperature is stable at this maximum only momentarily and falls rapidly because ice crystallization causes concentration of solutes which leads to a further depression of freezing point. The observed freezing point of milk (maximum temperature after initiation of crystallization) is not the same as its true freezing point since some ice crystallization will have occurred before the maximum temperature is reached. Correction factors have been suggested to account for this but, in practice, it is usual to report
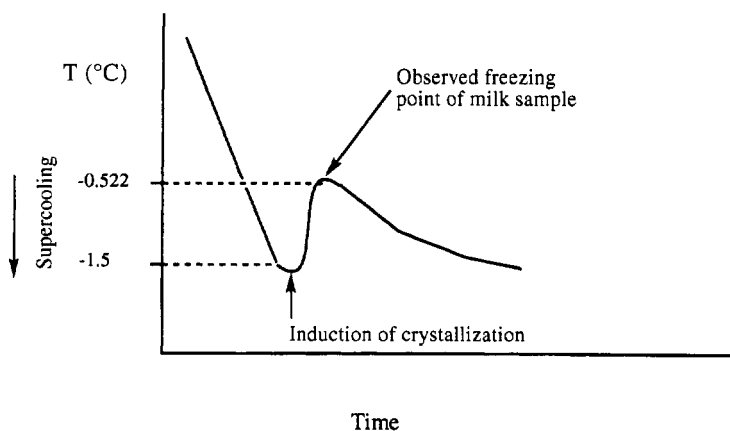

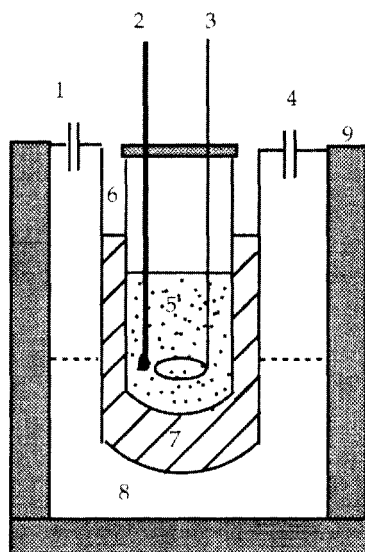Figure 11.4 Temperature–time curve for the freezing of milk.

Figure 11.5 Schematic representation of a Hortvet cryoscope. 1,4, Inlet and outlet for air or vacuum supply; 2, thermometer calibrated at 0.001°C intervals; 3, agitator; 5, milk sample; 6, glass tube; 7, alcohol; 8, ether cooled by evaporation; 9, insulated jacket.

the observed freezing point when other factors (particularly the degree of supercooling) have been standardized. Therefore, the observed freezing point of milk is empirical and great care is necessary to standardize methodology.

The Hortvet technique (originally described in 1921) has been used widely to estimate the freezing point of milk. The original apparatus consisted of a tube, containing the milk sample and a thermometer calibrated at 0.001°C intervals, which was placed in ethanol in a Dewar flask which was cooled indirectly by evaporation of ether (caused by pulling or pumping air through the ether, Figure 11.5). This apparatus has been modified to include mechanical refrigeration and various stirring or tapping devices to initiate crystallization. The early Hortvet cryoscopes used thermometers calibrated in degrees Hortvet (°H; values in °H are about 3.7% lower than in °C). The difference between °H and °C originates from differences in the freezing points of sucrose solutions measured using the Hortvet cryoscope and procedure and their true freezing points. IDF (1983) suggested the following formulae to interconvert °H and °C:

$$°C = 0.96418°H + 0.00085$$

$$°H = 1.03711°C - 0.00085$$

However, it is now recommended that thermometers be calibrated in °C. More recently, thermistors have been used instead of mercury thermometers. Cryoscopes based on dew point depression have also been approved for use. These latter instruments also use thermistors and are based on changes in osmotic pressure. Thermistor cryoscopes are now used more widely than Hortvet instruments.

Measurement of the freezing point depression of milk is used to estimate the degree of adulteration of milk with added water. Assuming an average freezing point of 0.550°C, the amount of added water can be calculated from:

$$\% \text{ added water} = \frac{0.550 - \Delta T}{0.550} \times (100 - \text{TS}) \qquad (11.11)$$

where ΔT is the observed freezing point depression of the test sample and TS is the % total solids in the milk. Interpretation of freezing point values when assaying milk suspected of being adulterated with water requires care. Milk with a freezing point of $-0.525°C$ or below is usually presumed to be unadulterated. Due to greater variation in the freezing point of milks drawn from individual animals than of bulk milk, specifications for the freezing point of bulk milk are more stringent than those for milks from individual animals. Finally, it should be noted that estimation of the adulteration of milk with water depends on the constancy of the freezing point (as discussed above). Adulteration of milk with isotonic solutions, e.g. ultrafiltration permeate (which is being considered for standardization of the protein content of milk, see Rattray and Jelen, 1996), will not be detected by this technique.

11.5 Interfacial tension

A phase can be defined as a domain bounded by a closed surface in which parameters such as composition, temperature, pressure and refractive index are constant but change abruptly at the interface. The principal phases in milk are its serum and fat and the most important interfaces are air/serum and fat/serum. If present, air bubbles, and ice, fat or lactose crystals will also constitute phases. Forces acting on molecules or particles in the bulk of a phase differ from those at an interface since the former are attracted equally in all directions while those at an interface experience a net attraction towards the bulk phase (Figure 11.6).

This inward attraction acts to minimize the interfacial area and the force which causes this decrease in area is known as the interfacial tension (γ). If one phase is air, the interfacial tension is referred to as surface tension. Interfacial tension can be expressed as force per unit length (N m^{-1}) or the energy needed to increase the interfacial area by a unit amount (J m^{-2} or N m^{-1}).
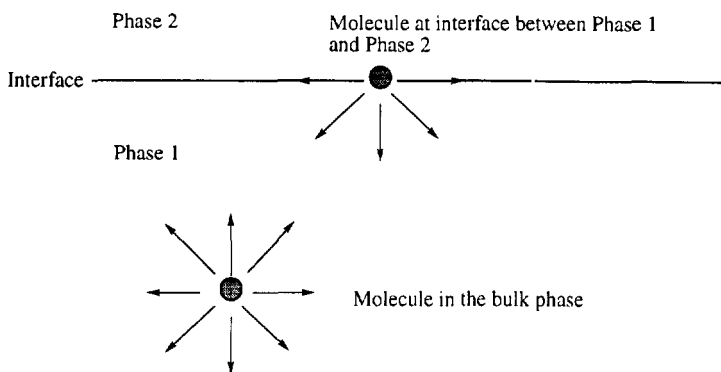
Figure 11.6 Schematic representation of the forces acting on a molecule or particle in a bulk phase or at an interface.

In addition to temperature (which decreases γ), the properties of interfaces are governed by the chemistry of the molecules present, their concentration and their orientation with respect to the interface. Solutes adsorbed at an interface which reduce interfacial tension are known as surface active agents or surfactants. Surfactants reduce interfacial tension by an amount given, under ideal conditions, by the Gibb's equation:

$$d\gamma = -RT\Gamma d\ln a \qquad (11.12)$$

where Γ is the excess concentration of the solute at the interface over that in the bulk solution, a is the activity of the solute in the bulk phase and R and T are the universal gas constant and temperature (in Kelvin), respectively. Therefore, the most effective surfactants are those which accumulate most readily at an interface.

Interfacial tension may be measured by a number of techniques, including determining how far a solution rises in a capillary, by measuring the weight, volume or shape of a drop of solution formed at a capillary tip, measuring the force required to pull a flat plate or ring from the surface or the maximum pressure required to form a bubble at a nozzle immersed in the solution. Ring or plate techniques are most commonly used to determine γ of milk.

Reported values for the interfacial tension between milk and air vary from 40 to $60 \, N \, m^{-1}$, with an average of about $52 \, N \, m^{-1}$ at 20°C (Singh, McCarthy and Lucey, 1997). At 20–40°C, the interfacial tension between milk serum and air is about $48 \, N \, m^{-1}$ while that between sweet cream, buttermilk and air is about $40 \, N \, m^{-1}$ (Walstra and Jenness, 1984). Surface tension values for rennet whey, skim milk and 25% fat cream are reported to be 51–52, 52–52.5 and $42–45 \, N \, m^{-1}$, respectively (Jenness and Patton, 1959).

The principal surfactants in milk are its proteins, phospholipids, mono- and diglycerides and salts of free fatty acids. The immunoglobulins are less effective surfactants than other milk proteins. Salts and lactose do not contribute significantly to the interfacial tension of milk. The difference in interfacial tension between milk serum/air and buttermilk/air can be attributed to the higher concentration of very surface active proteins and protein–phospholipid complexes of the fat globule membrane in buttermilk. The interfacial tension between milk fat globules and the milk serum is about $2 \, N \, m^{-1}$, while the interfacial tension between non-globular, liquid milk fat and milk serum is about $15 \, N \, m^{-1}$, indicating the effectiveness of milk fat globule membrane material in reducing interfacial tension. The surface tension of whole milk is a little lower than that of skim milk, possibly due to the presence of higher levels of material from the fat globule membrane and traces of free fat in the former. Surface tension decreases with increasing fat content up to about 4%. Lipolysis reduces the surface tension of milk due to the liberation of free fatty acids and attempts have been made to estimate hydrolytic rancidity by exploiting this fact, although such approaches have not been very successful (see Sherbon (1988) for references).

In addition to its composition, various processing parameters can influence the surface tension of milk. The surface tension of whole and skim milk decreases with increasing temperature. Surface tension also varies with the temperature history and age of the milk and with the time required for measurement. Homogenization of raw milk reduces surface tension because lipolysis by the indigenous milk lipase is stimulated and surface-active fatty acids released. Homogenization of pasteurized milk causes a slight increase in surface tension. Pasteurization of milk has little effect on its surface tension although heating milk to sterilization temperatures causes a slight increase in surface tension, resulting from denaturation and coagulation of proteins which are then less effective as surfacants.

11.6 Acid–base equilibria

The acidity of a solution is normally expressed as its pH, which may be defined as:

$$pH = -\log_{10} a_{H^+} \tag{11.13}$$

or

$$pH = -\log_{10} f_H [H^+] \tag{11.14}$$

where a_{H^-} is the activity of the hydrogen ion, $[H^+]$ its concentration and f_H its activity coefficient. For many dilute solutions, $f_H \approx 1$ and pH can thus be closely approximated by the negative logarithm of the hydrogen ion concentration.

The pH of milk at 25°C is usually in the range 6.5–6.7, with a mean value of 6.6. The pH of milk is influenced much more by temperature than is the pH of dilute buffers, principally due to the temperature dependence of the solubility of calcium phosphate (Chapter 5). pH varies with stage of lactation; colostrum can have a pH as low as 6.0. Mastitis tends to increase the pH since increased permeability of the mammary gland membranes means that more blood constituents gain access to the milk; the pH of cow's blood is 7.4. The difference in pH between blood and milk results from the active transport of various ions into the milk, precipitation of colloidal calcium phosphate (CCP; which results in the release of H^+) during the synthesis of casein micelles, higher concentrations of acidic groups in milk and the relatively low buffering capacity of milk between pH 6 and 8 (Singh, McCarthy and Lucey, 1997).

An important characteristic of milk is its buffering capacity, i.e. resistance to changes in pH on addition of acid or base. A pH buffer resists changes in the $[H^+]$ (ΔpH) in the solution and normally consists of a weak acid (HA) and its corresponding anion (A^-, usually present as a fully dissociatable salt). An equilibrium thus exists:

$$HA \rightleftharpoons H^+ + A^- \tag{11.15}$$

The addition of H^+ to this solution favours the back reaction while the addition of base favours the forward reaction. The weak acid/salt pair thus acts to minimize ΔpH. An analogous situation exists for buffers consisting of a weak base and its salt. The pH of a buffer can be calculated from the concentration of its components by the Henderson–Hasselbalch equation

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{11.16}$$

where pK_a is the negative logarithm of the dissociation constant of the weak acid, HA. A weak acid/salt pair is most effective in buffering against changes in pH when the concentrations of acid and salt are equal, i.e. at $pH = pK_a$ of HA. The effectiveness of a buffer is expressed as its buffering index

$$\frac{dB}{dpH} \tag{11.17}$$

Milk contains a range of groups which are effective in buffering over a wide pH range. The principal buffering compounds in milk are its salts (particularly soluble calcium phosphate, citrate and bicarbonate) and acidic and basic amino acid side-chains on proteins (particularly the caseins). The contribution of these components to the buffering of milk was discussed in detail by Singh, McCarthy and Lucey (1997).

In theory, it should be possible to calculate the overall buffering properties of milk by combining the titration curves for all components but in

practice this is not done since K_a values for many milk constituents are uncertain. Titration curves obtained for milk are very dependent on the technique used, and forward and back titrations may show a marked hysteresis in buffering index (Figure 11.7a). The buffering curve for milk titrated from pH 6.6 to pH 11.0 (Figure 11.7b) shows decreasing buffering from pH 6.6 to about pH 9. Milk has good buffering capacity at high pH values (above pH 10), due principally to lysine residues and carbonate anions. When milk is back titrated from pH 11.0 to pH 3.0, little hysteresis is apparent (Figure 11.7b). Buffering capacity increases below pH 6.6 and reaches a maximum around pH 5.1. This increase, particularly below pH 5.6, is a consequence of the dissolution of CCP. The resulting phosphate anions buffer against a decrease in pH by combining with H^+ to form HPO_4^{2-} and $H_2PO_4^-$. If an acidified milk sample is back titrated with base (Figure 11.7a), buffering capacity is low at about pH 5.1 and the maximum in the buffering curve occurs at a higher pH value (about 6.3), due to the formation of CCP from soluble calcium phosphate with the concomitant release of H^+. Ultrafiltration (UF) causes a steady increase in the buffering capacity of UF retentates due to increased concentrations of caseins, whey proteins and colloidal salts and makes it difficult to obtain an adequate decrease in pH during the manufacture of cheese from UF retentates.

Acid–base equilibria in milk are influenced by processing operations. Pasteurization causes some change in pH due to the loss of CO_2 and precipitation of calcium phosphate. Higher heat treatments (above $100°C$) result in a decrease in pH due to the degradation of lactose to various organic acids, especially formic acid (Chapter 9). Slow freezing of milk causes a decrease in pH since the formation of ice crystals during slow freezing concentrates the solutes in the aqueous phase of milk, with the precipitation of calcium phosphate and a concomitant release of H^+. Rapid freezing does not have this effect since there is insufficient time for the above changes to occur. Concentration of milk by evaporation of water causes a decrease in pH as the solubility of calcium phosphate is exceeded, resulting in the formation of more colloidal calcium phosphate. Conversely, dilution causes colloidal calcium phosphate to go into solution, with a corresponding decrease in $[H^+]$ (Chapter 5).

The buffering capacity of milk is often estimated by determining its titratable acidity, which involves titrating a sample of milk, containing a suitable indicator (usually phenolphthalein), with NaOH and thus is a measure of the buffering capacity of the milk between its natural pH and the phenolphthalein endpoint (i.e. between about pH 6.6 and 8.3). Titratable acidity is normally used to estimate the freshness of milk and to monitor the production of lactic acid during fermentation. Fresh milk typically requires 1.3–2.0 milliequivalents OH^- to titrate 100 ml from pH 6.6 to pH 8.3 (13–20 ml of 0.1 M NaOH), i.e. fresh milk has a titratable acidity of 0.14 to 0.16%, expressed as lactic acid.
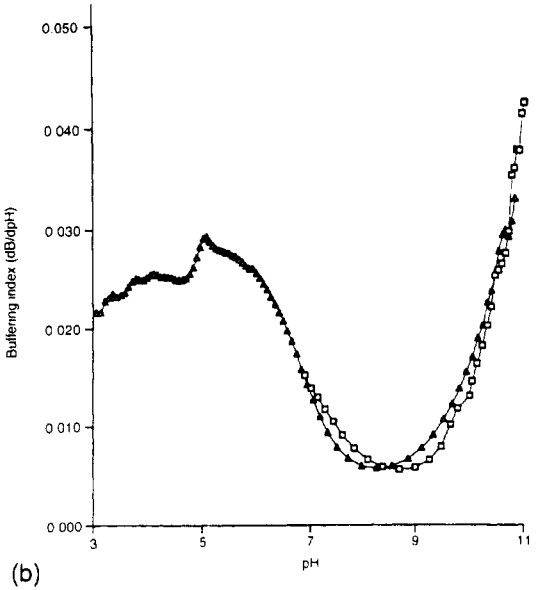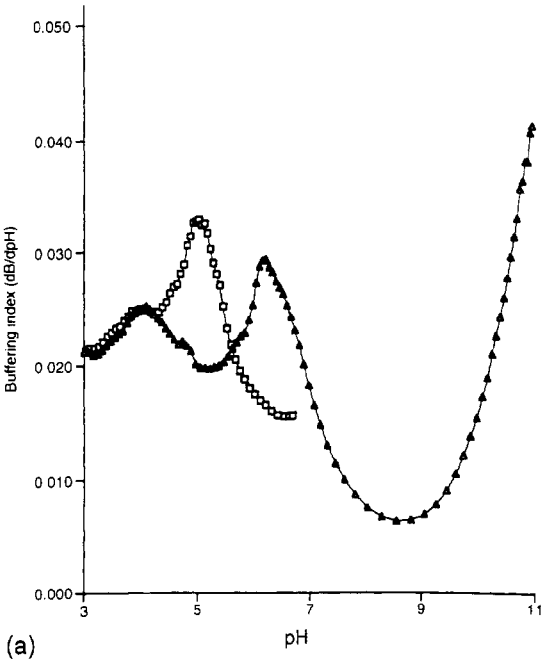
(a)

(b)

Figure 11.7 (a) Buffering curves of milk titrated from its initial pH (6.6) to pH 3.0 with 0.5 N HCl [□] and back-titrated to pH with 11.0 with 0.5 N NaOH [△].(b) Buffering curves of milk titrated from its initial pH (6.6) to pH 11.0 with 0.5 N NaOH [□] and back-titrated to pH with 3.0 with 0.5 N HCl [△]. (From Singh, McCarthy and Lucey, 1997.)

A high titratable acidity for fresh milk suggests high concentrations of proteins and/or other buffering constituents. Titratable acidity varies only slightly with the breed of cow, although the values for individual cows can vary more widely (0.08–0.25% as lactic acid). The liberation of fatty acids on lipolysis can interfere with the estimation of titratable acidity in high-fat products. Precipitation of calcium phosphate (with a concomitant decrease in pH) and 'fading of the phenolphthalein end-point' can occur during titration and thus the titratable acidity value obtained is influenced by the speed of titration.

11.7 Rheological properties

11.7.1 Newtonian behaviour

Under certain conditions (e.g. moderate shear rates, at fat contents below 40% and at temperatures above 40°C, at which the fat is liquid and no cold agglutination occurs) milk, skim milk and cream are, in effect, fluids with Newtonian rheological properties. Newtonian behaviour can be described by the equation:

$$\tau = \eta \dot{\gamma} \tag{11.18}$$

where τ is the shear stress (force per unit area, Pa), $\dot{\gamma}$ the shear rate (rate of change of velocity across the stream, s^{-1}) and η is the coefficient of viscosity (Pa s). The coefficient of viscosity for a Newtonian fluid is independent of shear rate but is influenced by temperature and pressure.

The coefficient of viscosity for whole milk at 20°C, but not affected by cold agglutination of fat globules, is about 2.127 mPa s. Values for water and milk plasma at 20°C are 1.002 and 1.68 mPa s, respectively. Casein, and to a lesser extent fat, are the principal contributors to the viscosity of milk; whey proteins and low molecular mass species have less influence.

The viscosity of milk and Newtonian milk products is influenced by composition, concentration, pH, temperature, thermal history and processing operations.

The Newtonian coefficient of viscosity at a given temperature for milk, creams and some concentrated milk products is related to the concentration of individual components by Eiler's equation:

$$\eta = \eta_0 \left(1 + \frac{1.25 \sum (\phi_i)}{1 - \sum (\phi_i)/\phi_{max}} \right)^2 \tag{11.19}$$

where η_0 is the coefficient of viscosity of the portion of the fluid consisting of water and low molecular mass species other than lactose and ϕ is the volume fraction of all dispersed particles that are at least an order of magnitude larger than water. The volume fraction of any component is

given by

$$\phi_i = V_i c_{v,i} \tag{11.20}$$

where V_i is the voluminosity of component i (in $m^3 kg^{-1}$ dry component) and $c_{v,i}$ is the volume concentration of the component in the product ($m^3 kg^{-1}$ product). The voluminosity of fat in fat globules is c. $1.11 \times 10^{-3} m^3 kg^{-1}$, that of casein micelles is c. $3.9 \times 10^{-3} m^3 kg^{-1}$, whey proteins c. $1.5 \times 10^{-3} m^3 kg^{-1}$ and lactose c. $1 \times 10^{-3} m^3 kg^{-1}$. For milk

$$\phi \approx \phi_f + \phi_c + \phi_w + \phi_l \tag{11.21}$$

where ϕ_f, ϕ_c, ϕ_w, ϕ_l are the volume fractions of fat, casein, whey proteins and lactose, respectively. ϕ_{max} is the assumed value of $\sum(\phi_i)$ for maximum packing of all dispersed particles (0.9 for fluid milk products).

Increasing pH increases viscosity slightly (perhaps as a consequence of micellar swelling) while a small decrease in pH reduces viscosity, although a large decrease in pH causes aggregation of casein micelles. Viscosity is inversely related to temperature. The viscosity of milk shows thermal hysteresis; it usually shows greater viscosity during heating than during subsequent cooling, probably due to the melting and crystallization behaviour of milk triglycerides.

The viscosity of milk and creams tends to increase slightly with age, due in part to changes in ionic equilibria. Heating skim milk to an extent that denatures most of the whey proteins increases its viscosity by about 10%. Homogenization of whole milk has little effect on its viscosity. The increase in the volume fraction of fat on homogenization is compensated by a decrease in the volume fractions of casein and whey proteins because some skim milk proteins are adsorbed at the fat–oil interface. Pasteurization has no significant effect on the rheology of whole milk.

11.7.2 Non-Newtonian behaviour

Raw milks and creams exhibit non-Newtonian rheological properties when they are held under conditions which favour cold agglutination of fat globules (below 40°C and low shear rates). Under such conditions, they show thixotropic (shear thinning) behaviour, i.e. their apparent viscosity (η_{app}) is inversely related to shear rate. Aggregates of fat globules and the milk serum trapped in their interstitial spaces have a large effective volume due to their irregular shapes. Increasing the shear rate causes increased shear stress to be applied to the aggregates which can disperse, yielding smaller or more rounded ones. Disaggregation reduces the interstitial space between fat globules, thereby reducing the total volume fraction of the fat phase and consequently reducing the η_{app} of the product. When the shearing force applied to the fluid increases in excess of the forces which hold the aggregates together, increases in shear rate cause increasingly smaller

changes in apparent viscosity. Thus, at high shear rates the fluid will exhibit Newtonian behaviour.

Increasing the fat content and/or reducing the temperature favours non-Newtonian behaviour. Low temperatures promote cold agglutination of fat globules and thus increase both η_{app} and deviation from Newtonian behaviour. The temperature at cream separation also influences the rheological properties of the resulting cream. Cream prepared by separation above 40°C shows less deviation from Newtonian behaviour since cryo-globulins are lost in the skim milk, resulting in less agglutination. Apparent viscosity is also influenced by the shear history of the product. The reformation of bonds between fat globules in aggregates requires time and thus the η_{app} versus shear rate ($\dot{\gamma}$) curves exhibit hysteresis. η_{app} increases after cessation of shearing (as aggregates are reformed) but usually does not return to its original value. Hysteresis is apparent in products containing aggregates caused by cold agglutination or homogenization.

Coalescence of fat globules does not change η_{app} since the volume fraction of the fat is not changed. However, partial coalescence can result in an increase in η_{app} due to entrapment of milk serum in aggregates. Indeed, high-fat creams can exhibit rheopectic (shear thickening) behaviour since shearing can cause partial coalescence of fat globules.

In addition to the general decrease in viscosity with increasing temperature, heating milk can also influence its rheology by heat-induced denaturation of cryoglobulins and/or other whey proteins. Concentration of milk, e.g. by ultrafiltration, prior to heating results in a greater increase in η_{app} than in milk heated before concentration.

The addition of hydrocolloids (e.g. carrageenans, pectins or carboxymethyl cellulose) as thickening agents will greatly increase the apparent viscosity of the product. The production of extracellular polysaccharides by certain bacteria will also increase the viscosity of milk products.

11.7.3   Rheology of milk gels

Gels are viscoelastic bodies, the rheological properties of which can be described by two parameters, the storage modulus (G', which is a measure of its elasticity) and the loss modulus (G'', which is a measure of its viscous nature). The combined viscoelastic modulus (G^*) is a measure of the overall resistance of a gel to deformation. These moduli are often highly dependent on the time-scale of deformation. Another important parameter of a food gel is its yield stress.

Although the gelation properties of whey proteins are of great importance in many foods (Mulvihill, 1992) and it is possible to form a weak gel in creams by the formation of a continuous network of fat globules, most important milk gels are those involving casein micelles which can be made to form a gel matrix either by isoelectric precipitation (acid-induced gel) or by the action of a proteolytic enzyme (rennet-induced gel). Both gel types

are relatively similar but, over long deformation times, rennet-induced gels have more liquid character than acid gels, which means that the former can flow under their own weight while acid gels are more likely to retain their shape. Rennet-induced gels also have a greater tendency to synerese and have a higher yield stress than acid-induced gels.

The firmness of acid- and rennet-induced milk gels is increased by such factors as time elapsed after aggregation of the micelles, gelation at elevated temperature, increasing casein and calcium phosphate concentrations and reduced pH (Walstra and Jenness, 1984). Heat-induced denaturation of whey proteins decreases the firmness of rennet-induced gels but increases the firmness of acid-induced gels. Fat globules weaken casein gels by interrupting the gel matrix. Casein molecules on the surface of fat globules in homogenized milk can participate in gel network formation. However, in practice this is influenced by a number of other factors, including preheating, homogenization pressure and temperature, and type of gel (Walstra and Jenness, 1984). Indeed, the yield stress of a rennet-induced milk gel may be reduced by homogenization.

11.7.4 Rheological properties of milk fat

The rheological properties of milk fat are greatly influenced by the ratio of solid to liquid fat and by the crystal form of the solid fat. At room temperature (20°C), milk fat is partially solid and has a plastic consistency, i.e. it exhibits viscoelastic properties; at small deformations (below 1%), it is almost completely elastic due to interactions between the fat crystals which form a weak network but it will begin to flow when subjected to greater deformations. As discussed by Walstra and Jenness (1984), the important parameters in determining the firmness of milk fat include the fraction of solid fat, the shape and size of fat crystals, heterogeneity throughout the fat and the extent to which fat crystals form a network throughout the mass of fat.

The structure of butter and other dairy spreads are further complicated by the presence of aqueous phase droplets and intact fat globules. Water droplets tend to weaken the structure and fat crystals inside intact fat globules cannot participate in the formation of a network thoughout the product (Chapter 3).

11.8 Electrical conductivity

The specific resistance (ρ, ohm cm) of a substance is related to its dimensions by:

$$\rho = \alpha R/l \tag{11.22}$$

where α is the cross-sectional area (cm^2), l is length (cm) and R the measured resistance (ohms). The specific conductance, K ($ohm^{-1} cm^{-1}$), is the reciprocal of specific resistance. The specific conductance of milk is usually in the range $0.0040-0.0055 ohm^{-1} cm^{-1}$. Ions (particularly Na^+, K^+ and Cl^-) are responsible for most of the electrical conductivity of milk which is increased by the bacterial fermentation of lactose to lactic acid. Measurement of the specific conductance of milk has been used as a rapid method for detecting subclinical mastitis. The conductivity of solutions is altered by concentration and dilution. However, the usefulness of this in the context of milk (e.g. to detect adulteration with water) is reduced considerably by the influence of concentration or dilution on the precipitation or solubilization of colloidal calcium phosphate. Direct conductivity measurements are thus unsuitable for assessing the amount of water added to milk.

11.9 Thermal properties of milk

The specific heat of a substance is the amount of heat energy, in kJ, required to increase the temperature of 1 kg of the substance by 1 K. The specific heat of skim milk increases from 3.906 to $3.993 kJ kg^{-1} K^{-1}$ from 1 to 50°C. Values of 4.052 and $3.931 kJ kg^{-1} K^{-1}$ have been reported for skim and whole milks, respectively, at 80°C (Sherbon, 1988). The specific heat of milk is inversely related to its total solids content, although discontinuities have been observed around 70–80°C. Skim-milk powder usually has a specific heat in the range $1.172-1.340 kJ kg^{-1} K^{-1}$ at 18–30°C.

The specific heat of milk fat (solid or liquid) is about $2.177 kJ kg^{-1} K^{-1}$. The specific heat of milk and cream is therefore strongly influenced by their fat content. Over most commonly encountered temperature ranges, the specific heat of high-fat dairy products is complicated by the latent heat absorbed by melting fat (about $84 J g^{-1}$). The observed specific heat of these products, at temperatures over which milk fat melts is thus the sum of the true specific heat and the energy absorbed to provide the latent heat of fusion of milk fat. Specific heat is thus influenced by factors such as the proportion of fat in the solid phase at the beginning of heating, and thus the composition of the fat and its thermal history. The apparent specific heat of high-fat dairy products (sum of 'true' specific heat and the energy absorbed by melting of fat) is usually maximal at 15–20°C and often has a second maximum or inflexion around 35°C.

The rate of heat transfer through a substance by conduction is given by the Fourier equation for heat conduction:

$$\frac{dQ}{dt} = -kA \frac{dT}{dx} \qquad (11.23)$$

where dQ/dt is the quantity of heat energy (Q) transferred per unit time (t), A is the cross-sectional area of the path of heat flow, dT/dx is the temperature gradient and k is the thermal conductivity of the medium. The thermal conductivity of whole milk (2.9% fat), cream and skim milk is roughly 0.559, 0.384 and $0.568 \, W \, m^{-1} \, K^{-1}$, respectively. The thermal conductivity of skim milk, whole milk and cream increases with increasing temperature but decreases with increasing levels of total solids or fat, particularly at higher temperatures. In addition to their composition, the thermal conductivity of dried-milk products depends on bulk density (weight per unit volume) due to differences in the amount of air entrapped in the powder.

Thermal diffusivity is a measure of the ability of a material to dissipate temperature gradients within it. Thermal diffusivity (α, $m^2 \, s^{-1}$) is defined as the ratio of thermal conductivity (k) to volumetric specific heat (density times specific heat, ρc):

$$\alpha = k/\rho c \tag{11.24}$$

The thermal diffusivity of milk (at 15–20°C) is about $1.25 \times 10^{-7} \, m^2 \, s^{-1}$.

11.10   Interaction of light with milk and dairy products

The refractive index (n) of a transparent substance is expressed by the relation:

$$n = \frac{\sin i}{\sin r} \tag{11.25}$$

where i and r are the angles between the incident ray and the refracted ray of light, respectively, and a perpendicular to the surface of the substance. The refractive index of milk is difficult to estimate due to light scattering by casein micelles and fat globules. However, it is possible to make accurate measurements of the refractive index of milk using refractometers in which a thin layer of sample is used, e.g. the Abbé refractometer. The refractive index of milk at 20°C using the D-line of the sodium spectrum ($\sim 589 \, nm$), n_D^{20}, is normally in the range 1.3440–1.3485. The refractive index of milk fat is usually in the range 1.4537–1.4552 at 40°C. Although there is a linear relationship between the solids content (weight per unit volume) and refractive index, determination of percentage solids in milk by refractometry is difficult, since the contributions of various milk components differ and are additive. The relationship between the refractive index of milk and its total solids content varies with changes in the concentration and composition of the solutes in milk. However, attempts have been made to measure the total contribution of solids and casein in milk and milk products by estimating

the refractive index (Sherbon, 1988). The specific refrative index (refractive constant), K, is calculated from:

$$K = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{\rho}$$ (11.26)

where n is the refractive index and ρ is density. Milk has a specific refractive index of about 0.2075.

Milk contains not only numerous dissolved chemical components but it is also an emulsion with a colloidal continuous phase. Therefore, milk absorbs light of a wide range of wavelengths and also scatters ultraviolet (UV) and visible light due to the presence of particles. Milk absorbs light of wavelengths between 200 and about 380 nm due to the proteins present and between 400 and 520 nm due to fat-soluble pigments (carotenoids). A number of functional groups in milk constituents absorb in the infrared (IR) region of the spectrum; the OH groups of lactose absorb at c. 9.61 μm, the amide groups of proteins at 6.465 μm and the ester carbonyl groups of lipids at 5.723 μm (Singh, McCarthy and Lucey, 1997). Since light scattering is reduced at longer wavelenghts in the IR region, the absorbance of IR light of specific wavelengths can be used to measure the concentrations of fat, protein and lactose in milk. Instruments using this principle are now widely used in the dairy industry. However, since milk contains about 87.5% water (which absorbs IR light strongly), it is opaque to light throughout much of the IR region of the spectrum.

Milk contains about 1.62 mg kg^{-1} riboflavin which fluoresces strongly on excitation by light of wavelenghts from 400 to 500 nm, emitting light with a λ_{max} = 530 nm. Milk proteins also fluoresce due to the presence of aromatic amino acid residues; part of the light absorbed at wavelengths around 280 nm is emitted at longer wavelengths.

Scattering of light by the colloidal fat particles present in milk has been used to estimate its fat content. A commercial apparatus (Milko-Tester^{TM}) has been developed which exploits this principle. Milk is diluted (to avoid multiple scatterings) using an EDTA solution which disperses the casein micelles. The milk sample is homogenized to ensure a uniform fat globule size and the extent of scattering of white light is determined.

11.11 Colour of milk and milk products

The white colour of milk results from scattering of visible light by casein micelles and fat globules. Homogenization of milk results in a whiter product due to increased scattering of light by smaller, homogenized, fat globules. The serum phase of milk is greenish due to the presence of riboflavin which is responsible for the characteristic colour of whey.
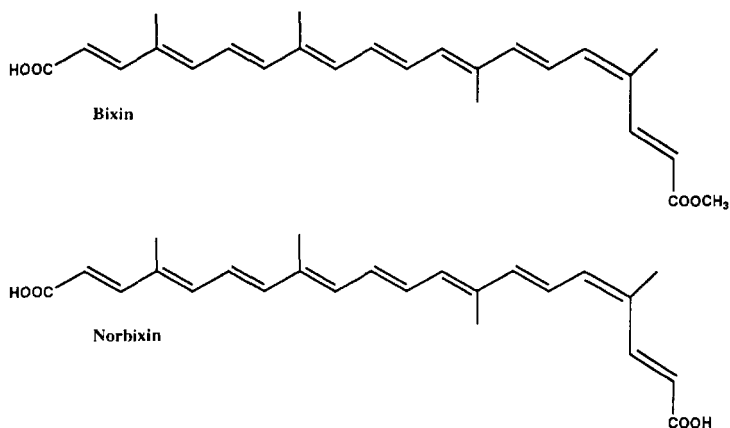
Figure 11.8 Structures of *cis*-bixin and norbixin, apocarotenoid pigments in annatto.

The colour of dairy products such as butter and cheese is due to fat-soluble pigments, especially carotenoids, which are not synthesized by the animal but are obtained from plant sources in the diet. Therefore, feed has a major effect on the colour of milk-fat. Cows fed on grass produce a more yellow-coloured fat than animals fed on hay or concentrates. The ability of cattle to metabolize carotenes to vitamin A varies between breeds and between individuals (Chapter 6).

The most widely used added colorant in dairy products is annatto (E160b) which is a yellow-orange preparation containing apocarotenoid pigments obtained form the pericarp of the seeds of the tropical shrub, *Bixa orellana*. The principal pigment in annatto is *cis*-bixin (methyl 9'-*cis*-6,6'-diapocarotene-6,6'-diooate) with smaller amounts of norbixin (*cis*-6,6'-diapocarotene-6,6'-dioic acid) (Figure 11.8). The heat treatment used in extraction normally converts *cis*-bixin to *trans*-bixin which is red and soluble in oil. Annatto is used to give a yellow colour to margarine and to colour 'red' Cheddar and other cheeses.

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